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1965

Biological Products

PUBLIC HEALTH SERVICE

REGULATIONS • TITLE 42 • PART 73



U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service

Biological Products

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PUBLIC HEALTH SERVICE REGULATIONS

Biological Products

Title 42 • Part 73

and

Excerpts from the Public Health Service Act

PUBLIC HEALTH SERVICE PUBLICATION NO. 437

Revised 1965

This publication is available from the Division of Biologics Standards
National Institutes of Health, Bethesda, Maryland, 20014

Public Health Service Publication No. 437
Revised October 1, 1965
Amendment No. 2

Federal Register
Vol. 31 p 9676
July 16, 1966

TITLE 42--PUBLIC HEALTH
CHAPTER 1--PUBLIC HEALTH SERVICE
DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
SUBCHAPTER F--QUARANTINE
INSPECTION, LICENSING
PART 73--BIOLOGICAL PRODUCTS
ADDITIONAL STANDARDS: WHOLE BLOOD (HUMAN)

On October 22, 1965, a notice of rule making was published in the Federal Register (30 F.R. 13456) proposing to amend Part 73 of the Public Health Service Regulations to include standards for the shipment of Whole Blood (Human) before results of certain tests are ascertained, to assure timely arrival for transfusion purposes.

Views and arguments respecting the proposed standards were invited to be submitted within 30 days after publication of the notice in the Federal Register, and notice was given of intention to make any amendments that were adopted effective 30 days after the date of their publication in the Federal Register.

After consideration of all comments submitted, the following amendments to Part 73 of the Public Health Service regulations

are hereby adopted to become effective 30 days after the date of publication in the Federal Register.

1. Amend §73.303(a) to read as follows:

§73.303 Testing the blood.

(a) Serological test for syphilis. Whole Blood (Human) shall be negative to a serological test for syphilis.

2. Amend §73.304 by deleting the present paragraph (f) and inserting a new paragraph (f) to read as follows:

§73.304 General requirements.

(f) Issue prior to determination of test results.

Notwithstanding the provisions of §73.70, blood may be issued by the licensee on the request of a physician, hospital or other medical facility, before results of all tests prescribed in §73.303 have been determined where such issue is essential to allow time for transportation to assure arrival of the blood by the time when needed for transfusion of such blood provided (1) the blood is shipped directly to such physician or medical facility, (2) the records of the licensee contain a full explanation of the need for such issue, (3) the label on each container of such blood bears the information required by §73.305(e), (4) the label does not bear results of tests other than those made on pilot samples of the blood to be shipped, taken at the time of its collection, and (5) the

Public Health Service Publication No. 437
Revised October 1, 1965
Amendment No. 1

Federal Register
Vol. 31 pp 14-15
January 4, 1966

TITLE 42--PUBLIC HEALTH
CHAPTER 1--PUBLIC HEALTH SERVICE
DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
SUBCHAPTER F--QUARANTINE
INSPECTION, LICENSING
PART 73--BIOLOGICAL PRODUCTS
ADDITIONAL STANDARDS: IMMUNE SERUM GLOBULIN (HUMAN)

On May 19, 1965 a notice of rule making was published in the Federal Register (30 F.R. 6795) proposing to amend Part 73 of the Public Health Service Regulations to include specific standards of safety, purity and potency for Immune Serum Globulin (Human).

Views and arguments respecting the proposed standards were invited to be submitted within 60 days after publication of the notice in the Federal Register, and notice was given of intention to make any amendments that were adopted effective 60 days after the date of their publication in the Federal Register.

After consideration of all comments submitted, the following amendment to Part 73 of the Public Health Service Regulations is hereby adopted to become effective 60 days

after the date of publication in the Federal Register.

1. Amend Part 73 of the Public Health Service Regulations by adding the following to the table of contents after "73.354 General requirements":

ADDITIONAL STANDARDS: IMMUNE SERUM GLOBULIN (HUMAN)

73.355 The product

73.356 Manufacture of Immune Serum Globulin (Human)

73.357 The final product

73.358 Potency

73.359 General requirements

2. Amend Part 73 of the Public Health Service Regulations by adding the following after §73.354:

ADDITIONAL STANDARDS: IMMUNE SERUM GLOBULIN (HUMAN)

§73.355 The product

(a) Proper name and definition. The proper name of this product shall be Immune Serum Globulin (Human). The product is defined as a sterile solution containing antibodies derived from human blood.

(b) Source material. The source of Immune Serum Globulin (Human) shall be blood, plasma or serum from human

donors determined at the time of donation to have been free of causative agents of diseases that are not destroyed or removed by the processing methods, as determined by the donor's history and from such physical examination and clinical tests as appear necessary for each donor at the time the blood was obtained. The source blood, plasma or serum shall not contain a preservative and shall be stored in a manner that will prevent contamination by microorganisms, pyrogens or other impurities.

(c) Additives in source material. Source blood, plasma or serum shall contain no additives other than citrate or acid citrate dextrose anticoagulant solution, unless it is shown that the processing method yields a product free of the additive to such an extent that the safety, purity and potency of the product will not be affected adversely.

§73.356 Manufacture of Immune Serum Globulin (Human).

(a) Processing method. The processing method shall be one that has been shown: (1) to be capable of concentrating tenfold from source material at least two different antibodies; (2) not to affect the integrity of the globulins; (3) to consistently yield a product which is safe for subcutaneous

and intramuscular injection and (4) not to transmit viral hepatitis.

(b) Microbial contamination. Low temperatures or aseptic techniques shall be used to minimize contamination by microorganisms. Preservatives to inhibit growth of microorganisms shall not be used during processing.

(c) Bulk storage. The globulin fraction may be stored in bulk prior to further processing provided it is stored in clearly identified hermetically closed vessels. Globulin as either a liquid concentrate or a solid and containing alcohol or more than 5 percent moisture, shall be stored at a temperature of -10° C. or lower. Globulin as a solid free from alcohol and containing less than 5 percent moisture, shall be stored at a temperature of 0° C. or lower.

(d) Determination of the lot. Each lot of Immune Serum Globulin (Human) shall represent a pooling of approximately equal amounts of material from not less than 1,000 donors.

(e) Sterilization and heating. The final product shall be sterilized promptly after solution. At no time during processing shall the product be exposed to temperatures above 45° C. and after sterilization the product shall not be exposed to temperatures above 30° to 32° C. for more than 72 hours.

§73.357 The final product.

(a) Final solution. The final product shall be a 16.5 ± 1.5 percent solution of globulin containing 0.3 molar glycine and a preservative.

(b) Protein composition. At least 90 percent of the globulin shall have an electrophoretic mobility not faster than -2.8×10^{-5} centimeters per volt per second, when measured at a 1 percent protein concentration in sodium diethylbarbiturate buffer at pH 8.6 and 0.1 ionic strength.

§73.358 Potency.

(a) Antibody levels and tests. Each lot of final product shall contain at least the minimum levels of antibodies for diphtheria, measles, and for at least one type of poliomyelitis. In the event the final bulk solution is stored at a temperature above 5° C. the antibody level tests shall be performed after such storage with a sample of the stored material.

(b) Minimum levels. The minimum antibody levels are as follows:

(1) No less than 2 units of diphtheria antitoxin per ml.

(2) A measles neutralizing antibody level of no less than 0.25 times the level of the reference measles serum, except that when recommended for use with Measles Virus Vaccine, Live, Attenuated, the measles antibody level shall be as prescribed in §73.353.

(3) A poliomyelitis neutralizing antibody level of no less than 1.0 for Type 1, 1.0 for Type 2, and 2.5 for Type 3, times the antibody level of the reference poliomyelitis immune globulin.

(c) Reference materials. The following reference materials shall be obtained from the Division of Biologics Standards:

(1) NIH reference measles serum for correlation of measles antibody titers.

(2) NIH reference poliomyelitis immune globulin for correlation of poliomyelitis antibody titers, Types 1, 2, and 3.

§73.359 General requirements.

(a) Heat stability test. Approximately 2 ml. of completely processed material of each lot shall not show

any visible sign of gelation after heating in a 12 x 75 mm. stoppered glass tube at 57° C. for four hours.

(b) Hydrogen ion concentration. The pH of final container material shall be 6.8 ± 0.4 when measured in a solution diluted to 1 percent protein with 0.15 molar sodium chloride.

(c) Turbidity. The product shall be free of turbidity as determined by visual inspection of final containers.

(d) Date of manufacture. The date of manufacture is the date of initiating the last valid measles or poliomyelitis antibody test (§73.358(b) (2) and (3)), whichever date is earlier.

(e) Labeling. In addition to complying with all applicable labeling required in this part, labeling shall indicate that:

(1) There is no prescribed potency for viral hepatitis antibodies.

(2) The product is not recommended for intravenous administration.

(3) The lot is or is not suitable for use with Measles Virus Vaccine, Live, Attenuated.

(4) The lot is or is not recommended for poliomyelitis.

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(f) Samples and protocols. For each lot of Immune Serum Globulin (Human) the following material shall be submitted to the Director, Division of Biologics Standards, National Institutes of Health, Bethesda, Md., 20014:

(1) A 50 ml. sample of the final product.

(2) All protocols relating to the history of each lot and all results of all tests prescribed in these additional standards.

(Sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216; interpret or apply sec. 351, 58 Stat. 702; 42 U.S.C. 262)

Dated: December 9, 1965.

[SEAL]

William H. Stewart, M.D.
Surgeon General.

Approved: December 23, 1965.

Wilbur J. Cohen,
Acting Secretary.

label does not bear the name ^{OR} any other identification of the intended recipient.

3. Amend §73.305 to read as follows:

§73.305 Labeling.

In addition to all other applicable labeling requirements, the following, except as prescribed in paragraph (e) of this section, shall appear on the label of each container:

(a) Anticoagulant. Quantity and kind of anticoagulant used and the volume of blood corresponding with the formula prescribed or approved under §73.302(d).

(b) Serological test. The serological test for syphilis used and the result.

(c) Blood group and type. Designation of blood group and Rh factors:

(1) The blood group and Rh factors shall be designated conspicuously.

(2) If a color scheme for differentiating the ABO blood groups is used, the color used to designate each blood group on the container shall be:

Blood Group A: Yellow

Blood Group B: Pink

Blood Group O: Blue

Blood Group AB: White

(d) Additional information for labels of Group O Bloods.

Each Group O blood shall be labeled with a statement indicating whether or not isoagglutinin titers or other tests to exclude so-called "dangerous" Group O bloods were performed, and indicating the classification based on such tests.

(e) Issue prior to determination of test results.

The label on each container of blood that is issued pursuant to the provisions of §73.304(f) shall bear the following information and instructions in lieu of the information specified in paragraphs (b), (c), and (d) of this section.

EMERGENCY SHIPMENT FOR USE ONLY BY

(Name of physician, hospital or other medical facility.)

CAUTION

BEFORE TRANSFUSION

1. Do not use until test results received from
(name of licensee).

2. Perform crossmatch.

(Sec. 215, 58 Stat. 690, as amended, 42 U.S.C. 216; interpret or apply sec. 351, 58 Stat. 702, 42 U.S.C. 262)

Dated: June 27, 1966.

[SEAL]

William H. Stewart, M.D.
Surgeon General.

Approved: July 7, 1966.

Wilbur J. Cohen,
Acting Secretary.

INTRODUCTION

This publication contains an unofficial reprint of (a) excerpts from the Public Health Service Act, as amended (42 U.S.C. 201 et seq.), covering those portions primarily applicable to the control of biological products and of (b) the regulations pertaining to biological products adopted pursuant to such Act (42 C.F.R. Part 73), effective as of October 1, 1965.

Interstate shipment and the export or import of biological products were originally subject to Federal control under the provisions of the Act of July 1, 1902; 32 Stat. 728, 729. In enacting the Public Health Service Act, this authority was consolidated with other laws relating to the Public Health Service by the Act of July 1, 1944, entitled "An Act to consolidate and revise the laws relating to the Public Health Service, and for other purposes" (Public Law 410, 78th Congress; 58 Stat. 682). Title III, Part F of the Public Health Service Act is the part that relates directly to the control of biological products.

The Public Health Service Regulations consist of Title 42, Chapter I of the Code of Federal Regulations. This chapter was rearranged and reprinted in its entirety in the Federal Register of September 16, 1947, to integrate the numerous amendments and additions issuing since the original codification of Title 42 on June 1, 1938. The present publication includes Part 73, Biological Products, of these Regulations and includes amendments published in the Federal Register since September 16, 1947.

Reorganization Plan No. 1, 1953 (67 Stat. 631), transferred the Public Health Service to the Department of Health, Education, and Welfare, abolished the Federal Security Agency and the Office of Federal Security Administrator, and transferred all functions of the Administrator to the Secretary of the Department. The Plan was made effective April 11, 1953, by the Act of April 1, 1953 (Public Law 13, 83rd Congress, 67 Stat. 18). Accordingly, for all actions subsequent to April 10, 1953, the references in the statute and regulations to the terms "Federal Security Agency," "Federal Security Administrator," or "Administrator" have respectively been changed in this reprint to "Department of Health, Education, and Welfare," "Secretary of the Department of Health, Education, and Welfare" and "Secretary."

EXCERPTS FROM THE PUBLIC HEALTH SERVICE ACT, As Amended

TITLE I—SHORT TITLE AND DEFINITIONS

SHORT TITLE

42 U.S.C., Note

SEC. 1. Titles I to VI inclusive, of this Act may be cited as the “Public Health Service Act.”¹

DEFINITIONS

42 U.S.C. 201

SEC. 2. When used in this Act—

(a) The term “Service” means the Public Health Service;

(b) The term “Surgeon General” means the Surgeon General of the Public Health Service;

(c) The term “Secretary” means the Secretary of the Department of Health, Education, and Welfare;

(d) The term “regulations,” except when otherwise specified, means rules and regulations made by the Surgeon General with the approval of the Secretary;

(e) The term “executive department” means any executive department, agency, or independent establishment of the United States or any corporation wholly owned by the United States;

(f) The term “State” means a State or the District of Columbia, Hawaii, Alaska, Puerto Rico, or the Virgin Islands, except that as used in section 361(d)² such term means a State, the District of Columbia, or Alaska;

(g) The term “possession” includes, among other possessions, Puerto Rico and the Virgin Islands.

TITLE II—ADMINISTRATION

PUBLIC HEALTH SERVICE

42 U.S.C. 202

SEC. 201. The Public Health Service in the Department of Health, Education, and Welfare shall be administered by the Surgeon General under the supervision and direction of the Secretary.

ORGANIZATION

42 U.S.C. 203

SEC. 202. The Service shall consist of (1) the Office of the Surgeon General, (2) the National Institutes³ of Health, (3) the Bureau of Medical Services, and

¹ Section 1 was amended by section 4 of the Hospital Survey and Construction Act to add Title VI to the original five titles of the Public Health Service Act.

² Relates to Part G—Quarantine and Inspection, Control of Communicable Diseases.

³ Section 202 was amended by 6(b) of the National Heart Act by adding an “s” to Institute.

(4) the Bureau of State Services. The Surgeon General is authorized and directed to assign to the Office of the Surgeon General, to the National Institutes of Health, to the Bureau of Medical Services, and to the Bureau of State Services, respectively, the several functions of the Service, and to establish within them such divisions, sections, and other units as he may find necessary; and from time to time abolish, transfer and consolidate divisions, sections, and other units and assign their functions and personnel in such manner as he may find necessary for efficient operation of the Service. No division shall be established, abolished, or transferred, and no divisions shall be consolidated, except with the approval of the Secretary. The National Institutes of Health shall be administered as a part of the field service. The Surgeon General may delegate to any officer or employee of the Service such of his powers and duties under this Act, except the making of regulations, as he may deem necessary or expedient.

TITLE III—GENERAL POWERS AND DUTIES OF PUBLIC HEALTH SERVICE

Part F—Biological Products

REGULATION OF BIOLOGICAL PRODUCTS

42 U.S.C. 262

SEC. 351. (a) No person shall sell, barter, or exchange, or offer for sale, barter, or exchange in the District of Columbia, or send, carry, or bring for sale, barter, or exchange from any State or possession into any other State or possession or into any foreign country, or from any foreign country into any State or possession, any virus, therapeutic serum, toxin, antitoxin, or analogous product, or arsphenamine or its derivatives (or any other trivalent organic arsenic compound), applicable to the prevention, treatment, or cure of diseases or injuries of man, unless (1) such virus, serum, toxin, antitoxin, or other product has been propagated or manufactured and prepared at an establishment holding an unsuspended and unrevoked license, issued by the Secretary as hereinafter authorized, to propagate or manufacture, and prepare such virus, serum, toxin, antitoxin, or other product for sale in the District of Columbia, or for sending, bringing, or carrying from place to place aforesaid; and (2) each package of such virus, serum, toxin, antitoxin, or other product is plainly marked with the proper name of the article contained therein, the name, address, and license number of the manufacturer, and the date beyond which the contents cannot be expected beyond reasonable doubt to yield their specific results. The suspension or revocation of any license shall not prevent the sale, barter, or exchange of any virus, serum, toxin, antitoxin, or other product aforesaid which has been sold and delivered by the licensee prior to such suspension or revocation, unless the owner or custodian of such virus, serum, toxin, antitoxin, or other product aforesaid has been notified by the Secretary not to sell, barter, or exchange the same.

(b) No person shall falsely label or mark any package or container of any virus, serum, toxin, antitoxin, or other product aforesaid; nor alter any label or mark on any package or container of any virus, serum, toxin, antitoxin, or other product aforesaid so as to falsify such label or mark.

(c) Any officer, agent, or employee of the Department of Health, Education, and Welfare, authorized by the Secretary for the purpose, may during all reasonable hours enter and inspect any establishment for the propagation or manufacture

and preparation of any virus, serum, toxin, antitoxin, or other product aforesaid for sale, barter, or exchange in the District of Columbia, or to be sent, carried, or brought from any State or possession into any other State or possession or into any foreign country, or from any foreign country into any State or possession.

(d) Licenses for the maintenance of establishments for the propagation or manufacture and preparation of products described in subsection (a) of this section may be issued only upon a showing that the establishment and the products for which a license is desired meet standards, designed to insure the continued safety, purity, and potency of such products, prescribed in regulations, and licenses for new products may be issued only upon a showing that they meet such standards. All such licenses shall be issued, suspended, and revoked as prescribed by regulations, and all licenses issued for the maintenance of establishments for the propagation or manufacture and preparation, in any foreign country, of any such products for sale, barter, or exchange in any State or possession shall be issued upon condition that the licensees will permit the inspection of their establishments in accordance with subsection (c) of this section.

(e) No person shall interfere with any officer, agent, or employee of the Service in the performance of any duty imposed upon him by this section or by regulations made by authority thereof.

(f) Any person who shall violate, or aid or abet in violating, any of the provisions of this section shall be punished upon conviction by a fine not exceeding \$500 or by imprisonment not exceeding one year, or by both such fine and imprisonment, in the discretion of the court.

(g) Nothing contained in this Act shall be construed as in any way affecting, modifying, repealing, or superseding the provisions of the Federal Food, Drug, and Cosmetic Act (U.S.C., 1940 edition, title 21, ch. 9).

PREPARATION OF BIOLOGICAL PRODUCTS

42 U.S.C. 263

Sec. 352. (a) The Service may prepare for its own use any product described in section 351 and any product necessary to carrying out any of the purposes of section 301.

(b) The Service may prepare any product described in section 351 for the use of other Federal departments or agencies, and public or private agencies and individuals engaged in work in the field of medicine when such product is not available from establishments licensed under such section.

PART 73—BIOLOGICAL PRODUCTS**STANDARDS FOR PRODUCTS: LABELS****DEFINITIONS**

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AUTHORITY: §§ 73.1-73.503 issued under sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216. Interpret or apply sec. 351, 58 Stat. 702; 42 U.S.C. 262. Other statutory provisions interpreted or applied are cited to text in parentheses.

CROSS REFERENCES: For Department of Health, Education, and Welfare regulations relating to drugs as defined in the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 et seq.), see 21 CFR, Subchapter C. For exemption from section 505 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 355) of new drugs licensed under the Public Health Service Act, see 21 CFR 130.2. For drugs intended solely for investigational use, see 21 CFR 1.114. For Bureau of Customs regulations relating to viruses, serums and toxins, see 19 CFR 12.21-12.23. For Post Office regulations relating to the admissibility to the United States mails see 39 CFR Parts 14 and 15, esp. § 15.(2).

DEFINITIONS

§ 73.1¹ Definitions.

As used in this part :

(a) "Act" means the Public Health Service Act (58 Stat. 682), approved July 1, 1944.

(b) "Secretary" means the Secretary of Health, Education, and Welfare.

(c) "Surgeon General" means the Surgeon General of the United States Public Health Service.

(d) "Institutes" means the National Institutes of Health in the Public Health Service.

(e) "Division of Biologics Standards" means the Division of Biologics Standards of the National Institutes of Health.

(f) "State" means a State or the District of Columbia, Puerto Rico, or the Virgin Islands.

(g) "Possession" includes among other possessions, Puerto Rico and the Virgin Islands.

(h) "Products" includes biological products and trivalent organic arsenicals.

(i) "Biologic product" means any virus, therapeutic serum, toxin, antitoxin, or analogous product applicable to the prevention, treatment or cure of diseases or injuries of man :

(1) ² A virus is interpreted to be a product containing the minute living cause of an infectious disease and includes but is not limited to filterable viruses, bacteria, rickettsia, fungi, and protozoa.

(2) ² A therapeutic serum is the product obtained from the blood of an animal by removing the clot or clot components and the blood cells, and not intended for ingestion.

(3) A toxin is a product containing a soluble substance poisonous to laboratory animals or to man in doses of 1 milliliter or less (or equivalent in weight) of the product, and having the property, following the injection of non-fatal doses into an animal, of causing to be produced therein another soluble substance which specifically neutralizes the poisonous substance and which is demonstrable in the serum of the animal thus immunized.

(4) An antitoxin is a product containing the soluble substance in serum or other body fluid of an immunized animal which specifically neutralizes the toxin against which the animal is immune.

(5) A product is analogous :

(i) To a virus if prepared from or with a virus or agent actually or potentially infectious, without regard to the degree of virulence or toxicogenicity of the specific strain used.

(ii) ² To a therapeutic serum, if composed of whole blood or plasma or containing some organic constituent or product other than a hormone or an amino acid, derived from whole blood, plasma, or serum, and not intended for ingestion.

(iii) ³ To a toxin or antitoxin, if intended, irrespective of its source of origin, to be applicable to the prevention, treatment, or cure of disease or injuries of man through a specific immune process.

(j) "Trivalent organic arsenicals" means arspenamine and its derivatives (or any other trivalent organic arsenic compound) applicable to the prevention, treatment, or cure of diseases or injuries of man.

(k) ³ A product is deemed "applicable to the prevention, treatment or cure of diseases or injuries of man" irrespective of the mode of administration or application recommended, including use when intended, through administration or application to a person, as an aid in diagnosis or in evaluating the degree of susceptibility or immunity possessed by a person, and including also any other use for purposes of diagnosis if the diagnostic substance so used is prepared from or with the aid of a biological product.

(l) "Proper name", as applied to a product, means the name designated in the license for use upon each container of the product.

(m) "Dating period" means the period beyond which the product cannot be expected beyond reasonable doubt to yield its specific results.

(n) ³ "Expiration date" means the time of termination of the dating period expressed as the calendar month, day, and year, and where applicable the hour at or in which the dating period ends.

(o) The word "standards" means specifications and procedures applicable to an establishment or to the manufacture or release of products, which are prescribed in this part and which are designed to insure the continued safety, purity and potency of such products.

¹ Sec. 73.1 amended by adding a new subsection (h) and redesignating subsections (h) through (aa) as (i) through (bb), 11/3/61, 26 FR 10355.

² Sec. 73.1(i) (1), (2), and (5)(ii) amended 11/3/61, 26 FR 10355.

³ Secs. 73.1(i) (5)(iii) ; 73.1(k) ; 73.1(n) ; and 73.1(q) amended, 11/3/61, 26 FR 10356.

(p) The word "continued" as applied to the safety, purity and potency of products is interpreted to apply to the dating period.

(q)¹ The word "safety" means the relative freedom from harmful effect to persons affected, directly or indirectly, by a product when prudently administered, taking into consideration the character of the product in relation to the condition of the recipient at the time.

(r) The word "sterility" is interpreted to mean freedom from viable contaminating microorganisms, as determined by the tests prescribed in § 73.73.

(s) "Purity" means relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product. "Purity" includes but is not limited to relative freedom from residual moisture or other volatile substances and pyrogenic substances.

(t) The word "potency" is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result.

(u) "Manufacturer" means any legal person or entity engaged in the manufacture of a product subject to license under the Act.

(v) "Manufacture" means all steps in propagation or manufacture and preparation of products and includes but is not limited to filling, testing, labeling, packaging, and storage by the manufacturer.

(w) "Location" includes all buildings, appurtenances, equipment and animals used, and personnel engaged by a manufacturer within a particular area designated by an address adequate for identification.

(x) "Establishment" includes all locations.

(y) "Lot" means that quantity of uniform material identified by the manufacturer as having been thoroughly mixed in a single container.

(z) A "filling" refers to a group of final containers identical in all respects, which have been filled with the same product from the same bulk lot without any change that will affect the integrity of the filling assembly.

(aa) "Process" refers to a manufacturing step that is performed on the product itself which may affect its safety, purity or potency, in contrast to such manufacturing steps which do not affect intrinsically the safety, purity or potency of the product.

(bb) "Selling agent" or "distributor" means any person engaged in the unrestricted distribution, other than by sale at retail, of products subject to license.

LICENSES: PROCEDURE

§ 73.2 Two forms of licenses.

There shall be two forms of licenses: establishment and product.

§ 73.3 Application for establishment and product licenses; procedure for filing.

To obtain a license for any establishment or product, the manufacturer shall make application to the Director, Division of Biologics Standards, on forms prescribed for such purpose, and in the case of an application for a product license, shall submit data derived from laboratory and clinical studies which demonstrate that the manufactured product meets prescribed standards of safety, purity and potency, a full description of manufacturing methods, data establishing stability of the product through the dating period, sample(s) representative of the product to be sold, bartered or exchanged or offered, sent, carried or brought for sale, barter or exchange, summaries of results of tests performed on the lot(s) represented by the submitted sample(s), and specimens of the labels, enclosures and containers proposed to be used for the product. An application for license shall not be considered as filed until all pertinent information and data shall have been received from the manufacturer by the Division of Biologics Standards.

§ 73.4 Establishment licenses; issuance and conditions.

(a) *Inspection—compliance with standards.* An establishment license shall be issued only after inspection of the establishment and upon a de-

¹ Secs. 73.1(i) (5) (iii); 73.1(k); 73.1(n); and 73.1(q) amended, 11/3/61, 26 FR 10356.

termination that the establishment complies with the applicable standards prescribed in the regulations in this part.

(b) *Availability of product; simultaneous request for and issuance of product license.* No establishment license shall be issued unless (1) a product intended for sale, barter or exchange or intended to be offered, sent, carried or brought for sale, barter or exchange is available for examination, (2) such product is available for inspection during all phases of manufacture and (3) a product license is requested and issued simultaneously with the establishment license.

(c) *One establishment license to cover all locations.* One establishment license shall be issued to cover all locations meeting the establishment standards.

§ 73.5 Product licenses; issuance and conditions.

(a) *Examination—compliance with standards.* A product license shall be issued only upon examination of the product and upon a determination that the product complies with the standards prescribed in the regulations in this part: *Provided*, That no product license shall be issued except upon a determination that the establishment complies with the establishment standards prescribed in the regulations contained in this part, applicable to the manufacture of such product.

(b) *Manufacturing process—impairment of assurances.* No product shall be licensed if any part of the process of or relating to the manufacture of such product, in the judgment of the Surgeon General, would impair the assurances of continued safety, purity and potency as provided by the regulations contained in this part.

§ 73.6 License forms.

(a) *Establishment license.* The establishment license form shall be prescribed by the Surgeon General and shall include:

- (1) The name and address of the manufacturer.
- (2) The name and address of the establishment.
- (3) The names and addresses of all locations of the establishment.
- (4) The license number.
- (5) The date of issuance.

(b) *Product license.* The product license form shall be prescribed by the Surgeon General and shall include:

- (1) The name and address of the manufacturer.
- (2) The name and address of the establishment.
- (3) The name and address of each location at which the product is manufactured.
- (4) The license number of the establishment.
- (5) The proper name of the product, with additional specifications, if any, which may be approved or required for additional labeling purposes.

§ 73.7 Changes to be reported.

Important changes in location, equipment, management and responsible personnel, or in manufacturing methods and labeling of any licensed product or of any product for which an application for a license is pending shall be immediately reported to the Director, Division of Biologics Standards, by any establishment holding a license, and, unless in case of an emergency, not less than 30 days in advance of the time such changes are made; failure to make such report shall constitute a ground for summary suspension of a license pending reinspection of the establishment or re-examination of the product.

§ 73.8 Products under development.

A biological product or trivalent organic arsenical undergoing development, but not yet ready for a product license, may be shipped or otherwise delivered from one State or possession into another State or possession provided such shipment or delivery is not for sale, barter or exchange and is in accordance with section 505 of the Federal Food, Drug, and Cosmetic Act, as amended, and the regulations thereunder.

§ 73.9¹ Issuance, revocation or suspension.

A license shall be issued by the Secretary upon the recommendation of the Surgeon General and upon the determination by the Surgeon General that the establishment or the product, as the case may be, meets the standards established by the reg-

¹ Sec. 73.9 amended June 28, 1961, 26 FR 5752, to become effective July 28, 1961.

ulations in this part as herein prescribed or hereafter amended. Licenses shall be valid until suspended or revoked. An establishment or product license shall be revoked upon application of the manufacturer giving notice of intention to discontinue the manufacture of all products or of intention to discontinue the manufacture of a particular product for which a license is held. The Surgeon General shall recommend to the Secretary that a license be suspended or revoked whenever he finds, after notice and opportunity for hearing, that

(a) Public Health Service inspectors after reasonable efforts have been unable to gain access to an establishment or a location for the purpose of carrying out the inspection required under § 73.31, or that (b) manufacturing of products or of a product has been discontinued to an extent that a meaningful inspection cannot be made, or (c) the establishment or any location thereof, or the product for which the license has been issued, fails to conform to the standards in the regulations in this part, as herein prescribed or as hereafter amended, designed to insure the continued safety, purity and potency of the manufactured product. In case of suspension, unless assurances satisfactory to the Surgeon General (a) that access will be permitted or (b) that manufacturing will be resumed, have been provided or (c) if the faulty condition is not corrected within 60 days or within such other period as may be specified in the notice of suspension, whichever is applicable, he shall recommend that the license be revoked. Except as provided in § 73.11, prior to the institution of proceedings looking to the suspension or revocation of a license the licensee shall be advised in writing of the facts or conduct which may warrant such action and shall be accorded opportunity within a reasonable period prescribed by the Surgeon General to demonstrate or achieve compliance with the regulations in this part.

§ 73.10 Licenses heretofore issued.

Any license heretofore issued and in effect upon the effective date of the regulations in this part shall remain in effect unless and until superseded

by a new license, or suspended or revoked, pursuant to the regulations in this part.

§ 73.11 Summary suspension.

Whenever the Surgeon General has reasonable ground to believe that an establishment or product for which a license has been issued fails to conform to the standards prescribed in the regulations in this part, and that by reason of such failure and of failure of the manufacturer to take prompt corrective measures on notice thereof, the distribution or sale of a licensed product would constitute a danger to health, or that the establishment and manufacturing methods have been so changed as to require in order to protect the public health a new showing that the establishment or product meets the standards prescribed in the regulations in this part, he may recommend to the Secretary that the license for the establishment or the product be summarily suspended and the manufacturer be required (a) to notify the selling agents and distributors to whom such product or products have been delivered of such suspension, (b) to furnish complete records of such deliveries and notice of suspension, and (c) to show cause within 60 days or such other period as may be specified in the order why the license should not be revoked.

§ 73.12 Review Board.

When deemed advisable by the Surgeon General, in matters involving the safety, purity and potency of licensed products or products for which an application for license is pending, the reports of inspection and laboratory examinations, together with any pertinent data the establishment may submit, shall be passed upon by a special board of three officers appointed by the Surgeon General for that purpose. The board shall report its findings to the Surgeon General who will forward its report, together with his findings and recommendations, to the Secretary.

§ 73.13 Opportunity for hearing.

Any manufacturer whose application for a license has been denied, or whose establishment or

product license has been summarily suspended, without prior opportunity for hearing, may appeal from such denial or suspension and shall be entitled to a hearing thereon before a review body constituted as provided in § 73.12. The Surgeon General, upon review of the record, may affirm, reverse, or modify the findings of the review board, or may direct the taking of further testimony, and shall forward his determinations and recommendations to the Secretary.

§ 73.14 Suspension and revocation; Publication.

Notice of suspension or revocation of license, with statement of cause therefor, may be published by the Secretary.

§ 73.15 Licenses; reissuance.

(a) *Compliance with standards.* An establishment or product license, previously suspended or revoked, whether upon application, or for failure to comply with standards or changes in standards prescribed in the regulations in this part, may be reissued or reinstated upon a showing of compliance with required standards and upon such inspection and examination as may be considered necessary by the Director of the Division of Biologics Standards.

(b) *Exclusion of noncomplying location.* An establishment or product license, excluding a location or locations that fail to comply with prescribed standards, may be issued without further application and concurrently with the suspension or revocation of the license for noncompliance at the excluded location or locations.

§ 73.16¹ Products in short supply; initial manufacturing at other than licensed establishment.

Licenses issued to a manufacturer for an establishment shall authorize persons other than

¹ Sec. 73.16 amended June 28, 1961, 26 FR 5755, to become effective July 28, 1961.

such manufacturer to conduct at places other than such establishment the initial, and partial manufacturing of a product for shipment solely to such manufacturer only to the extent that the names of such persons and places are registered with the Surgeon General and he finds, upon application of such manufacturer, that (a) the product is in short supply due either to the peculiar growth requirements of the organism involved or to the scarcity of the animal required for manufacturing purposes, and (b) such manufacturer has established with respect to such persons and places such procedures, inspections, tests or other arrangements as will assure full compliance with the applicable regulations of this part related to continued safety, purity and potency. Such persons and places shall be subject to all regulations of this part except §§ 73.2 to 73.15, 73.20 to 73.23, and 73.50 to 73.55. Failure of such manufacturer to maintain such procedures, inspections, tests or other arrangements, or failure of any person conducting such partial manufacturing to comply with applicable regulations shall constitute a ground for summary suspension or revocation of the authority conferred pursuant to this section on the same basis as provided in §§ 73.11, 73.13 and 73.14 with respect to the summary suspension and the revocation of licenses.

FOREIGN ESTABLISHMENTS AND PRODUCTS

§ 73.20 Licenses required; products for controlled investigation only.

Any biological or trivalent organic arsenical manufactured in any foreign country and intended for sale, barter or exchange shall be refused entry by collectors of customs unless manufactured in an establishment holding an unsuspended and unrevoked establishment license and license for the product. Unlicensed products which are not imported for sale, barter or exchange and which are intended solely for purposes of controlled investigation are admissible only if in accord with section 505 of the Federal Food, Drug, and Cosmetic Act, as amended, and the regulations thereunder.

§ 73.21 Procedure.

Except as otherwise provided in this part, licenses for foreign establishments and products shall be issued, suspended or revoked in the same manner as licenses for domestic establishments and products. Each foreign establishment holding a license and consigning any licensed biological product or trivalent organic arsenical into any State or possession shall be required to file with the Surgeon General the name and address of any representative or representatives authorized by the establishment to distribute the product and such representative or representatives shall keep such records of such distribution as are required of domestic licensed establishments, and failure to maintain such records shall constitute ground for revocation of license.

§ 73.22 Form of license.

Licenses for establishments located in foreign countries shall be in form similar to that for domestic establishments except that they shall authorize manufacture for sending, carrying, or bringing for sale, barter or exchange from the foreign country designated in the license into any State or possession of the United States and shall specify that it is issued upon the condition that the licensee will permit the inspection during all reasonable hours of the establishment by any officer, agent, or employee of the Department of Health, Education, and Welfare authorized by the Secretary of the Department of Health, Education, and Welfare for such purpose.

§ 73.23 Samples to accompany each importation.

Each foreign importation of a biological product or trivalent organic arsenical from a licensed establishment, whether or not intended for investigational use only, shall be accompanied by at least two final containers of each lot of any biological product and by at least 15 final containers of each lot of any trivalent organic arsenical contained in the shipment. Such samples shall be forwarded by the Collector of Customs at the port of entry to the Director, Division of Biologics Standards for examination. If separate samples are not

found accompanying the shipment, samples shall be obtained from the shipment by the Collector of Customs and forwarded to the Director, Division of Biologics Standards.

(Sec. 801, 52 Stat. 1058, as amended; 21 U.S.C. 381)

ESTABLISHMENT INSPECTION**§ 73.30 Inspectors.**

Inspections shall be made by an officer of the Public Health Service having special knowledge of the methods used in the manufacture and control of products and designated for such purpose by the Surgeon General or by any officer, agent, or employee of the Department of Health, Education, and Welfare specifically designated for such purpose by the Secretary.

§ 73.31 Time of inspection.

The inspection of an establishment for which a license is pending need not be made until the establishment is in operation and is manufacturing the complete product for which a product license is desired. In case the license is denied following inspection for the original license, no reinspection need be made until assurance has been received that the faulty conditions which were the basis of the denial have been corrected. An inspection of each licensed establishment shall be made at least once each year. Inspections may be made with or without notice, and shall be made during regular business hours unless otherwise directed.

§ 73.32¹ Duties of inspector.

The inspector shall:

- (a) Call upon the active head of the establishment, stating the object of his visit,
- (b) Interrogate the proprietor or other personnel of the establishment as he may deem necessary,
- (c) Examine the details of location, construction, equipment and maintenance, including stables, barns, warehouses, manufacturing laboratories, bleeding clinics maintained for the collection of human blood, shipping rooms, record

¹ Secs. 73.32 and 73.35 amended June 28, 1961, 26 FR 5752, and 5755, to become effective July 28, 1961.

rooms, and any other structure or appliance used in any part of the manufacture of a product,

(d) Investigate as fully as he deems necessary the methods of propagation, processing, testing, storing, dispensing, recording, or other details of manufacture and distribution of each licensed product, or product for which a license has been requested, including observation of these procedures in actual operation,

(e) Obtain and cause to be sent to the Director, Division of Biologics Standards, adequate samples for the examination of any product or ingredient used in its manufacture,

(f) Bring to the attention of the manufacturer any fault observed in the course of inspection in location, construction, manufacturing methods, or administration of a licensed establishment which might lead to impairment of a product,

(g) Inspect and copy, as circumstances may require, any records required to be kept pursuant to § 73.37.

(h) Certify as to the condition of the establishment and of the manufacturing methods followed and make recommendations as to action deemed appropriate with respect to any application for license or any license previously issued.

ESTABLISHMENT STANDARDS

§ 73.35¹ Personnel.

(a) *Responsible head.* A person shall be designated as the responsible head who shall exercise control of the establishment in all matters relating to compliance with the provisions of this part, with authority to represent the manufacturer in all pertinent matters with the Division of Biologics Standards, and with authority to enforce or to direct the enforcement of discipline and the performance of assigned functions by employees engaged in the manufacture of products. The responsible head shall have an understanding of the scientific principles and the techniques involved in the manufacture of products. The responsible head shall have the responsibility for the training of employees in manufacturing methods and for their being informed concerning the application

of the pertinent provisions of this part to their respective functions.

(b) *Other personnel.* Personnel shall have capabilities commensurate with their assigned functions, a thorough understanding of the manufacturing operations which they perform, the necessary training and experience relating to individual products, and adequate information concerning the application of the pertinent provisions of this part to their respective functions. Personnel shall include such professionally trained persons as are necessary to insure the competent performance of all manufacturing processes.

(c) *Restrictions on personnel*—(1) *Specific duties.* Persons whose presence can affect adversely the safety and purity of a product shall be excluded from the room where the manufacture of a product is in progress.

(2) *Sterile operations.* Personnel performing sterile operations shall wear clean or sterilized protective clothing and devices to the extent necessary to protect the product from contamination.

(3) *Pathogenic viruses and spore-bearing organisms.* Persons working with viruses pathogenic for man or with spore-bearing microorganisms, and persons engaged in the care of animals or animal quarters, shall be excluded from areas where other products are manufactured, or such persons shall change outer clothing, including shoes, or wear protective covering prior to entering such areas.

(4) *Live vaccine work areas.* Persons may not enter a live vaccine processing area after having worked with other infectious agents in any other laboratory during the same working day. Only persons actually concerned with propagation of the culture, production of the vaccine, and unit maintenance, shall be allowed in live vaccine processing areas when active work is in progress. Casual visitors shall be excluded from such units at all times and all others having business in such areas shall be admitted only under supervision. Street clothing, including shoes, shall be replaced or covered by suitable laboratory clothing before entering a live vaccine processing unit. Persons caring for animals used in the manufacture of live vaccines shall be excluded from other animal quarters and from contact with other animals during the same working day.

¹ Secs. 73.32 and 73.35 amended June 28, 1961, 26 FR 5752, and 5755, to become effective July 28, 1961.

§ 73.36¹ Physical establishment, equipment, animals and care.

(a) *Work areas.* All rooms and work areas where products are manufactured or stored shall be kept orderly, clean, and free of dirt, dust, vermin and objects not required for manufacturing. Precautions shall be taken to avoid clogging and back-siphonage of drainage systems. Precautions shall be taken to exclude extraneous infectious agents from manufacturing areas. Work rooms shall be well lighted and ventilated. The ventilation system shall be arranged so as to prevent the dissemination of microorganisms from one manufacturing area to another and to avoid other conditions unfavorable to the safety of the product. Filling rooms, and other rooms where open, sterile operations are conducted, shall be adequate to meet manufacturing needs and such rooms shall be constructed and equipped to permit thorough cleaning and to keep air-borne contaminants at a minimum. If such rooms are used for other purposes, they shall be cleaned and prepared prior to use for sterile operations. Refrigerators, incubators and warm rooms shall be maintained at temperatures within applicable ranges and shall be free of extraneous material which might affect the safety of the product.

(b) *Equipment.* Apparatus for sterilizing equipment and the method of operation shall be such as to insure the destruction of contaminating microorganisms. The effectiveness of the sterilization procedure shall be no less than that achieved by an attained temperature of 121.5° C. maintained for twenty minutes by saturated steam or by an attained temperature of 170° C. maintained for two hours with dry heat. Processing vessels, storage containers, filters, filling apparatus and other pieces of apparatus and accessory equipment, including pipes and tubing, shall be designed and constructed to permit thorough cleaning and, where possible, inspection for cleanliness. All surfaces that come in contact with products shall be clean and free of extraneous material. For products for which sterility is a factor, equipment shall be sterile unless sterility of the product is assured by subsequent procedures.

(c) *Laboratory and bleeding rooms.* Rooms

used for the processing of products, including bleeding rooms, shall be effectively fly-proofed and kept free of flies and vermin. Such rooms shall be so constructed as to insure freedom from dust, smoke and other deleterious substances and to permit thorough cleaning and disinfection. Rooms for animal injection and bleeding, and rooms for smallpox vaccine animals, shall be disinfected and be provided with the necessary water, electrical and other services.

(d) *Animal quarters and stables.* Animal quarters, stables and food storage areas shall be of appropriate construction, fly-proofed, adequately lighted and ventilated, and maintained in a clean, vermin-free and sanitary condition. No manure or refuse shall be stored as to permit the breeding of flies on the premises, nor shall the establishment be located in close proximity to off-property manure or refuse storage capable of engendering fly breeding.

(e) *Restrictions on building and equipment use—*(1) *Work of a diagnostic nature.* Laboratory procedures of a clinical diagnostic nature involving materials that may be contaminated, shall not be performed in space used for the manufacture of products, except that manufacturing space which is used only occasionally may be used for diagnostic work provided spore-bearing pathogenic microorganisms are not involved and provided the space is thoroughly cleaned and disinfected before the manufacture of products is resumed.

(2) *Spore-bearing organisms for supplemental sterilization procedure control test.* Spore-bearing organisms used as an additional control in sterilization procedures may be introduced into areas used for the manufacture of products, only for the purposes of the test and only immediately before use for such purposes: *Provided, That* (i) the organism is not pathogenic for man and does not produce pyrogens or toxins, (ii) the organism does not grow at or below 37° C. within a two-week period, (iii) the culture is demonstrated to be pure, (iv) test cultures are not transferred to culture media in areas used for the manufacture of products, (v) each culture be labeled with the name of the microorganism and the statement "Caution: microbial spores. See directions for storage, use and disposition", and (vi) the con-

¹ Sec. 73.36 redesignated (formerly 73.37) and amended June 28, 1961, 26 FR 5752, to become effective July 28, 1961.

tainer of each such culture is designed to withstand handling without breaking.

(3) *Work with spore-bearing organisms.* Except as provided in the previous paragraph, all work with spore-bearing microorganisms shall be done in an entirely separate building: *Provided*, That such work may be done in a portion of a building used in the manufacture of products not containing spore-bearing microorganisms if such portion is completely walled-off and is constructed so as to prevent contamination of other areas and if entrances to such portion are independent of the remainder of the building. All containers, apparatus and equipment used for spore-bearing microorganisms shall be permanently identified and reserved exclusively for use with those organisms. Materials destined for further manufacturing may be removed from such an area only under conditions which will prevent the introduction of spores into other manufacturing areas.

(4) *Live vaccine processing.* Specific space which may be individual or multiple units shall be set aside for work with each live vaccine. Such space shall not be used for any other purpose during the processing period for that vaccine. Each such unit shall be isolated either in a separate building, in a separate wing of a building, or in quarters at the blind end of a corridor so situated as to be an independent unit. The separate unit or units in which live vaccine is processed shall include adequate space and equipment for all processing steps up to filling into final containers. Test procedures which potentially involve the presence of microorganisms other than the vaccine strains, or the use of tissue culture cell lines other than primary cultures, shall not be conducted in live vaccine processing areas.

(f) *Animals used in manufacture—(1) Care of animals used in manufacturing.* Caretakers and attendants for animals used for the manufacture of products shall be sufficient in number and have adequate experience to insure adequate care. Animal quarters and cages shall be kept in sanitary condition. Animals on production shall be inspected daily to observe response to production procedures. Animals that become ill for reasons not related to production shall be isolated from other animals and shall not be used for production until recovery is complete. Competent veterinary care shall be provided as needed.

(2) *Quarantine of animals.* Animals used in production shall have been kept under competent daily inspection and preliminary quarantine for a period of at least 7 days before use, or as otherwise provided in this part for specific products. Only healthy animals free from detectable communicable diseases shall be used for production. Particular care shall be taken during the quarantine periods to reject animals of the equine genus which may be infected with glanders and animals which may be infected with tuberculosis.

(3) *Immunization against tetanus.* Horses and other animals susceptible to tetanus, that are used in the manufacture of biological products, except those which are under active immunization for the manufacture of tetanus antitoxin, shall receive injections of tetanus toxoid in such amounts and at such intervals as experience has shown adequate to insure immunity to tetanus.

(4) *Immunization and bleeding of animals used as a source of products.* Toxins or other non-viable antigens used in the immunization of production animals shall be sterile. When used in the immunization of production animals, antigens containing viable microorganisms shall be free of extraneous contamination as determined by suitable tests prior to use. Injections shall not be made into horses within six inches of bleeding site. Horses shall not be bled for manufacturing purposes while showing persistent general reaction or local reaction near the site of bleeding. Blood shall not be used for manufacturing if it was drawn within 5 days of injecting the animal with viable microorganisms. Animals shall not be bled for manufacturing purposes when they have an intercurrent disease. Blood intended for use as a source of a biological product shall be collected in clean, sterile containers. When the product is intended for use by injection, such containers shall also be pyrogen-free.

(5) *Smallpox vaccine production animals.* Animals used for the manufacture of smallpox vaccine shall be thoroughly cleaned with soap and water at the beginning of the quarantine and at its conclusion. The animals shall not be vaccinated in areas most likely to be contaminated with feces.

(6) *Reporting of certain diseases.* In cases of actual or suspected infection with foot and mouth disease, glanders, tetanus, anthrax, gas gangrene, equine infectious anemia, equine encephalomye-

litis, or any of the pock diseases among animals intended for use or used in the manufacture of products, the manufacturer shall immediately notify the Director, Division of Biologics Standards.

(g) *Filling procedures.* Filling procedures shall be such as will not affect adversely the safety, purity or potency of the product.

(h) *Containers and closures.* All final containers and closures shall be made of material that will not hasten the deterioration of the product or otherwise render it less suitable for the intended use. All final containers and closures shall be clean and free of surface solids, and of leachable contaminants and other materials that will hasten the deterioration of the product or otherwise render it less suitable for the intended use. After filling, sealing shall be performed in a manner that will maintain the integrity of the product during the dating period. In addition, final containers and closures for products intended for use by injection shall be sterile and free from pyrogens. Final containers for products intended for use by injection shall be colorless and sufficiently transparent to permit visual examination of the contents under normal light.

§ 73.37¹ Records.

(a) *Maintenance of records.* Records shall be made, concurrently with the performance, of each step in the manufacture and distribution of products, in such a manner that at any time successive steps in the manufacture and distribution of any lot may be traced by an inspector. Such records shall be legible and indelible, shall identify the person immediately responsible, shall include dates of the various steps, and be as detailed as necessary for clear understanding of each step by one experienced in the manufacture of products.

(b) *Records retention*—(1) *General.* Records shall be retained for such interval beyond the expiration date as is necessary for the individual product, to permit the return of any clinical report of unfavorable reactions. The retention period shall be no less than five years after the records of manufacture have been completed or six months

after the latest expiration date for the individual product, whichever represents a later date.

(2) *Records of recall.* Complete records shall be maintained pertaining to the recall from distribution of any product upon notification by the Director, Division of Biologics Standards, to recall for failure to conform with the standards prescribed in the regulations of this part, because of deterioration of the product or for any other factor by reason of which the distribution of the product would constitute a danger to health.

(3) *Suspension of requirement for retention.* The Director, Division of Biologics Standards, may authorize the suspension of the requirement to retain records of a specific manufacturing step upon a showing that such records no longer have significance for the purposes for which they were made: *Provided*, That a summary of such records shall be retained.

(c) *Records of sterilization of equipment and supplies.* Records relating to the mode of sterilization, date, duration, temperature and other conditions relating to each sterilization of equipment and supplies used in the processing of products shall be made by means of automatic recording devices or by means of a system of recording which gives equivalent assurance of the accuracy and reliability of the record. Such records shall be maintained in a manner that permits an identification of the product with the particular manufacturing process to which the sterilization relates.

(d) *Animal necropsy records.* A necropsy record shall be kept on each animal from which a biological product has been obtained and which dies or is sacrificed while being so used.

(e) *Records in case of divided manufacturing responsibility.* If two or more establishments participate in the manufacture of a product, the records of each such establishment must show plainly the degree of its responsibility. In addition, each participating manufacturer shall furnish to the manufacturer who prepares the product in final form for sale, barter or exchange, a copy of all records relating to the manufacturing operations performed by such participating manufacturer insofar as they concern the safety, purity and potency of the lots of the product involved, and the manufacturer who prepares the product in final form shall retain a complete record of all

¹ Sec. 73.37 redesignated (formerly 73.36) and amended June 28, 1961, 26 FR 5753, to become effective July 28, 1961.

the manufacturing operations relating to the product.

§ 73.38¹ Retention samples.

Manufacturers shall retain for a period of at least six months after the expiration date, a quantity of representative material of each lot of each product, sufficient for examination and testing for safety and potency, except Whole Blood (Human), Packed Red Blood Cells, Single Donor Plasma, Antihemophilic Plasma, Normal Human Plasma and Allergenic extracts prepared to physician's prescription. Samples so retained shall be selected at random from either final container material, or from bulk and final containers, provided they include at least one final container as a final package, or package-equivalent of such filling of each lot of the product as intended for distribution. Such sample material shall be stored at temperatures and under conditions which will maintain the identity and integrity of the product. Samples retained as required in this section shall be in addition to samples required in this part of specific products to be submitted to the Division of Biologics Standards. Exceptions may be authorized by the Director, Division of Biologics Standards, when the lot yields relatively few final containers and when such lots are prepared by the same method in large numbers and in close succession.

STANDARDS FOR PRODUCTS: LABELS

§ 73.50 Container labels.

(a) The following items shall appear on the label affixed to each container of a product capable of bearing a full label:

- (1) The proper name of the product;
- (2) Name, address, and license number of manufacturer;
- (3) Lot number;
- (4) The expiration date.

(b) If the final container is capable of bearing only a partial label, the final container shall show as a minimum the name (expressed either as the

proper or common name), the lot number, and the name of the manufacturer and, if the final container is incapable of bearing any label, the items shall appear only on the outside label.

(c) If the final container is a multiple dose container, the container label must indicate the recommended dose. When the label has been affixed to the container a sufficient area of the container must remain uncovered for its full length or circumference to permit inspection of the contents.

§ 73.51 Proper name on outside label.

The proper name in the form designated in the product license for such purpose must appear upon the outside label in legible type and shall be given precedence in position and prominence over any trade-mark or trade name used:

(a) The "outside label" is the label of the carton enclosing one or more final containers, except that if no such carton is used the label of the individual final container is regarded as the outside label.

(b) "Legible type" includes only type of a size and character which can be read with ease when held in a good light and with normal vision.

(c) "Precedence in position" of the proper name will have been observed if it is placed above any trade-mark or trade name and provided it is symmetrically arranged with respect to other printing on the label.

(d) "Precedence in prominence" of the proper name will have been observed if the style of type is of the same or greater point size and of equal face, or heavier, than that used in printing the trade-mark or trade name, and if the contrast in color value between the proper name and the background is not less than that between the trade-mark or trade name and the background.

§ 73.52 Outside label; additional items.

The label affixed to the outside carton shall include, in addition to the proper name and the items required on the label of the final container, the following:

- (a) The preservative used and its concentration,
- (b) The volume of the contents, if a liquid, or the weight, if a solid, and the potency or dosage if more than one strength is dispensed,

¹ Sec. 73.38 redesignated (formerly 73.36(e)) and amended June 28, 1961, 26 FR 5754, to become effective July 28, 1961.

- (c) The recommended storage temperature,
- (d) The words "Shake Well," or equivalent, when indicated by the character of the product,
- (e) The dose and route of administration recommended or reference to such directions in an enclosed circular,
- (f) The source of the product when a factor in safe administration,
- (g) Minimum potency of product expressed in terms of official standard of potency or, if potency is a factor and no standard of potency has been prescribed, the words "No U.S. standard of potency."

§ 73.53¹ Divided manufacturing responsibility to be shown.

If two or more establishments participate in the manufacture of a product, the name, address, and license number of each must appear on the label of the final container, if capable of bearing a full label, and on the outside label.

§ 73.54 Name of selling agent or distributor.

The name and address of the selling agent or distributor of a product may appear on the label under the designation of "selling agent" or "distributor" provided that the name and address of the manufacturer is given precedence in prominence.

§ 73.55 Products for export.

Labels on packages or containers of products for export may be adapted to meet specific requirements of the regulations of the country to which the product is to be exported provided that in all such cases the minimum label requirements prescribed in § 73.50 are observed.

STANDARDS FOR PRODUCTS: GENERAL

§ 73.70¹ Tests prior to release required for each lot.

No lot of any licensed product shall be released by the manufacturer prior to the completion of tests for conformity with standards applicable to

such product. Each applicable test shall be made on each lot after completion of all processes of manufacture which may affect compliance with the standard to which the test applies. The results of all tests performed shall be considered in determining whether or not the test results meet the test objective, except that a test result may be disregarded when it is established that the test is invalid due to causes unrelated to the product.

§ 73.71 Potency.

Tests for potency shall consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in § 73.1(t).

§ 73.72¹ General safety.

In addition to specified safety tests prescribed in this part for individual products, a general safety test shall be performed in final container material, from each filling of each lot of all products intended for administration to man, either after the labels have been affixed to the final container, or affixed, both outside and inside, to the multiple container storage receptacle just prior to its sealing for storage purposes. Exceptions to this procedure may be authorized by the Director, Division of Biologics Standards, when more than one lot is processed each day. The general safety test shall consist of the parenteral injection of the maximum volume tolerated into each of two mice weighing approximately 20 gms. each and into each of two guinea pigs weighing approximately 350 gms. each but no more than 0.5 ml. need be inoculated into each mouse and no more than 5.0 ml. need be inoculated into each guinea pig. After injection the animals shall be observed for a period of no less than seven days and if neither significant symptoms nor death results during the observation period, the product meets the requirements for general safety. Variations of this test, either in the volume injected or in the species of test animal used shall be made whenever required because of the human dose level demanded of the product or because of any individual demands of the product itself.

¹ Secs. 73.53, 73.70 and 73.72 amended June 28, 1961, 26 FR 5754, to become effective July 28, 1961.

§ 73.73 Sterility.

Except as provided in paragraph (f), the sterility of each lot of each product shall be demonstrated by the performance of the tests prescribed in paragraphs (a) and (b) of this section for both bulk and final container material. Bulk material shall be tested separately from final container material and material from each final container shall be tested in individual test vessels.

(a)¹ *The test*—(1) *Using Fluid Thioglycollate Medium.* The volume of product, as required by paragraph (d) of this section (hereinafter referred to also as the “inoculum”), from samples of both bulk and final container material, shall be inoculated into test vessels of Fluid Thioglycollate Medium. The inoculum and medium shall be mixed thoroughly and incubated at a temperature of 30° to 32° C. for a test period of no less than seven days and examined visually for evidence of growth on the third or fourth or fifth day and on the seventh or eighth day. If incubation is continued beyond eight days, an additional examination shall be made on the last day of the test period. If the inoculum renders the medium turbid so that the absence of growth cannot be determined reliably by visual examination, portions of this turbid medium in amounts of no less than 1.0 ml. shall be transferred on the third or fourth or fifth day of incubation, from each of the test vessels and inoculated into additional vessels of medium. The material in the additional vessels shall be incubated at a temperature of 30° to 32° C. for no less than seven days. Notwithstanding such transfer of material, examination of the original vessels shall be continued as prescribed above. The additional test vessels shall be examined visually for evidence of growth on the third or fourth or fifth day of incubation and on the seventh or eighth day and if incubation is continued beyond a period of eight days, an additional examination shall be made on the last day of the incubation period. If growth appears, repeat tests may be performed as prescribed in paragraph (b) of this section and interpreted as specified in paragraph (c) of this section.

(2) *Using Fluid Sabouraud Medium.* Except for dried products, a test for fungi and yeast shall

be made on final container material, following the procedures prescribed in subparagraph (1) of this paragraph except that (i) the medium shall be Fluid Sabouraud Medium; (ii) the incubation shall be at a temperature of 20° to 25° C.; (iii) the period of incubation shall be no less than ten days and an examination shall be made on the tenth or eleventh or twelfth day in lieu of an examination on the seventh or eighth day.

(b) *Repeat tests*—(1) *Repeat bulk test.* If growth appears in the test of the bulk material, the test may be repeated to rule out faulty test procedures by testing at least the same volume of material.

(2) *First repeat final container test.* If growth appears in any test (thioglycollate or Sabouraud) of final container material, that test may be repeated to rule out faulty test procedures by testing material from a sample of at least the same number of final containers.

(3) *Second repeat final container test.* If growth appears in any first repeat final container test (thioglycollate or Sabouraud), that test may be repeated provided there was no evidence of growth in any test of the bulk material and material from a sample of twice the number of final containers used in the first test is tested by the same method used in the first test.

(c)² *Interpretation of test results.* The results of all tests performed on a lot shall be considered in determining whether or not the lot meets the requirements for sterility, except that tests may be excluded when demonstrated by adequate controls to be invalid. The lot meets the test requirements if no growth appears in the tests prescribed in paragraph (a) of this section. If repeat tests are performed, the lot meets the test requirements if no growth appears in the tests prescribed in paragraph (b)(2) or (b)(3) of this section, whichever is applicable.

(d)² *Test samples and volumes*—(1) *Bulk.* Each sample for the bulk sterility test shall be representative of the bulk container material and the volume tested shall be no less than 10 ml. (Note exceptions in paragraph (f) of this section.)

(2) *Final containers.* The sample for the final container and first repeat final container tests shall be no less than 20 final containers from each filling

¹ Sec. 73.73(a) amended January 11, 1962, 27 FR 307, to become effective February 10, 1962.

² Sec. 73.73 (c) and (d) amended January 11, 1962, 27 FR 307, to become effective February 10, 1962.

of each lot, selected to represent all stages of filling from the bulk container. If the amount of material in the final container is 1.0 ml. or less, the entire contents shall be tested. If the amount of material in the final container is more than 1.0 ml., the volume tested shall be the largest single dose recommended by the manufacturer or 1.0 ml., whichever is larger, but no more than 10 ml. of material or the entire contents from a single final container need be tested. (Note exceptions in paragraph (f) of this section.)

(e) *Culture medium*—(1) *Formulae*. (i) The formula for Fluid Thioglycollate Medium is as follows:

Fluid Thioglycollate Medium

1-cystine	0.5 gm.
Sodium chloride.....	2.5 gm.
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	5.5 gm.
Granular agar (less than 15% moisture by weight).	0.75 gm.
Yeast extract (water-soluble)	5.0 gm.
Pancreatic digest of casein.....	15.0 gm.
Purified water.....	1,000.0 ml.
Sodium thioglycollate (or thioglycollic acid—0.3 ml.).	0.5 gm.
Resazurin (0.10% solution, freshly prepared) ..	1.0 ml.
Final pH 7.1 ± 0.1 .	

(ii) The formula for Fluid Sabouraud Medium is as follows:

Fluid Sabouraud Medium

Dextrose	20 gm.
Pancreatic digest of casein.....	5 gm.
Peptic digest of animal tissue.....	5 gm.
Purified water.....	1,000 ml.
Final pH 5.7 ± 0.1 .	

(2) *Culture medium requirements*—(i) *Quality and condition of medium and design of container*. The growth promoting qualities and conditions of the culture medium, and the design of the test container, shall be such as are shown to provide conditions favorable to aerobic and anaerobic growth of microorganisms throughout the test period.

(ii) *Ratio of inoculum to culture medium*. The ratio of the volume of the inoculum to the volume of culture medium shall be such as will dilute the preservative in the inoculum to a level that does not inhibit growth of contaminating microorganisms. Inhibitors or neutralizers of preservative may be considered in determining the proper ratio.

(f) *Exceptions*. Bulk and final container material shall be tested for sterility as described above in this section except as follows:

(1) *Different sterility tests prescribed*. When different sterility tests are prescribed for a product in this part.

(2) *Alternate incubation temperatures*. Two tests may be performed, in all respects as prescribed in paragraph (a) (1) of this section, one test using an incubation temperature of 18° to 22° C., the other test using an incubation temperature of 35° to 37° C., in lieu of performing one test using an incubation temperature of 30° to 32° C.

(3) *Different tests equal or superior*. A different test may be performed provided that prior to the performance of such test a manufacturer submits data which the Surgeon General finds adequate to establish that the different test is equal or superior to the tests described in paragraphs (a) and (b) of this section in detecting contamination and makes the finding a matter of official record.

(4) *Test precluded or not required*. The tests prescribed in this section need not be performed for Whole Blood (Human), Packed Red Blood Cells (Human), Single Donor Plasma (Human), Smallpox Vaccine and other similar products concerning which the Surgeon General finds that the mode of administration, the method of preparation or the special nature of the product precludes or does not require a sterility test.

(5) *Viscous biological products*. Thioglycollate Broth Medium may be used in lieu of Fluid Thioglycollate Medium to test viscous biological products. The formula for Thioglycollate Broth Medium is as follows:

Thioglycollate Broth Medium. Certain biological products are turbid or otherwise do not lend themselves readily to culturing in Fluid Thioglycollate Medium because of its viscosity. In such instances, the following broth is acceptable in place of the Fluid Thioglycollate Medium, provided it is used in Smith fermentation tubes which have been heated within four hours in a boiling water bath or in free-flowing steam so as to drive the dissolved oxygen out of the medium in the closed arm:

1-cystine	0.5 gm.
Sodium chloride.....	2.5 gm.
Dextrose ($C_6H_{12}O_6 \cdot H_2O$)	5.5 gm.
Yeast extract (water-soluble)	5.0 gm.
Pancreatic digest of casein.....	15.0 gm.

Purified water----- 1,000.0 ml.
 Sodium thioglycollate (or thioglycolic acid—0.3 ml.). 0.5 gm.
 Final pH 7.1 ± 0.1 .

(6) *Number of final containers more than 20, less than 200.* If the number of final containers in the filling is more than 20 or less than 200, the sample shall be no less than 10 percent of the containers.

(7) *Number of final containers—20 or less.* If the number of final containers in a filling is 20 or less, the sample shall be two final containers, or the sample need be no more than one final container, provided (i) the bulk material met the sterility test requirements and (ii) after filling, it is demonstrated by testing a simulated sample that all surfaces to which the product was exposed were free of contaminating microorganisms. The simulated sample shall be prepared by rinsing the filling equipment with sterile 1.0 percent peptone solution, pH 7.1 ± 0.1 , which shall be discharged into a final container by the same method used for filling the final containers with the product.

(8)¹ *Samples—large volume of product in final containers.* For Normal Serum Albumin (Human), Normal Human Plasma, Antihemophilic Plasma (Human), Plasma Protein Solution (Human), and Fibrinogen (Human), when the volume of product in the final container is 50 ml. or more, the final containers selected as the test sample may contain less than the full volume of product in the final containers of the filling from which the sample is taken: *Provided*, That the containers and closures of the sample are identical with those used for the filling to which the test applies and the sample represents all stages of that filling.

(9) *Diagnostic products not intended for injection.* For diagnostic products not intended for injection, (i) only the Thioglycollate Medium test is required, (ii) the volume of material for the bulk test shall be no less than 2.0 ml., and (iii) the sample for the final container test shall be no less than three final containers if the total number filled is 100 or less, and, if greater, one additional container for each additional 50 containers or fraction thereof, but the sample need be no more than 10 containers.

¹ Sec. 7373(f)(8) amended and a new subparagraph (f)(10) added January 11, 1962, 27 FR 307, to become effective February 10, 1962.

(10)¹ *Human immune globulin preparations.* For human immune globulin preparations, the test samples from the bulk material and from each final container need be no more than two ml.

§ 73.74² Purity.

Products shall be free from extraneous material. In addition, products shall be tested as provided in paragraphs (a) and (b) of this section.

(a) *Test for residual moisture.* Each lot of dried product shall be tested for residual moisture and other volatile substances.

(1) *Procedure.* The test for dried products shall consist of measuring the maximum loss of weight in a weighed sample equilibrated over anhydrous P_2O_5 at a pressure of not more than one mm. of mercury, and at a temperature of 20° to 30° C. for as long as it has been established is sufficient to result in a constant weight.

(2) *Test results; standard to be met.* The residual moisture and other volatile substances shall not exceed one percent except as otherwise provided in this part: *Provided*, That for Thrombin, Fibrinogen, Streptokinase, Streptokinase-Streptodornase, and Anti-Influenza Virus Serum for the Hemagglutination-Inhibition Test, they shall not exceed three percent.

(b) *Test for pyrogenic substances.* Each lot of any product intended for use by injection shall be tested for pyrogenic substances by intravenous injection into rabbits as provided in subparagraphs (1) and (2) of this paragraph: *Provided*, That notwithstanding any other provision of this part, the test for pyrogenic substances is not required for the following products: Products containing formed blood elements; Single Donor Plasma (Human); Normal Horse Serum; Normal Rabbit Serum; bacterial, viral and rickettsial vaccines and antigens; toxoids; toxins; allergenic extracts; venoms; diagnostic substances and trivalent organic arsenicals.

(1) *Test dose.* The test dose for each rabbit shall be at least three milliliters per kilogram of body weight of the rabbit and also shall be at least equivalent proportionately, on a body weight basis, to the maximum single human dose recommended, except that: (i) Regardless of the human dose recommended, the test dose per kilogram of

² Sec. 73.74 amended June 30, 1960, 25 FR 6314, to become effective August 29, 1960.

body weight of each rabbit shall be, at least one milliliter for immune globulins derived from human blood, at least three milliliters for Normal Human Plasma, and at least 30 milligrams for Fibrinogen (Human); (ii) for Streptokinase, Streptokinase-Streptodornase and Radioiodinated (131) Serum Albumin (Human) the test dose shall be at least equivalent proportionately on a body weight basis to the maximum single human dose recommended.

(2) *Procedure.* Products shall be tested for freedom from pyrogenic substances by intravenous injection of the test dose into three or more rabbits in overt good health and by recording for each rabbit a control temperature taken within one hour prior to injection, and three additional temperatures taken one, two, and three hours after injection. For purposes of subparagraph (3) of this paragraph, if there is no temperature increase over the control temperature (i.e. where the temperature remains unchanged or falls), the temperature rise shall be considered as zero. If there is an increase in temperature over the control temperature, the temperature rise shall be the difference between the highest of the three hourly readings and the control temperature reading.

(3) *Test results; standards to be met.* The results recorded for all rabbits used in all tests of a lot of a product shall be included in determining whether the standard for purity is met. The product fails to meet test requirements if one-half or more of all rabbits show a temperature rise of 0.6° C. or more or if the average temperature rise of all rabbits is 0.5° C. or more.

(c) *Different tests equal or superior.* A different test for residual moisture may be performed provided that prior to its performance the manufacturer submits data which the Surgeon General finds adequate to establish that the different test is equal or superior to the test described in paragraph (a) of this section and makes the finding a matter of official record.

(Sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216. Interpret or apply sec. 351, 58 Stat. 702, as amended; 42 U.S.C. 262)

§ 73.75¹ Identity.

The contents of a final container of each filling of each lot shall be tested for identity after all

labeling operations shall have been completed. The identity test shall be specific for each product in a manner that will adequately identify it as the product designated on final container and package labels and circulars, and distinguish it from any other product being processed in the same laboratory. Identity may be established either through the physical or chemical characteristics of the product, inspection by macroscopic or microscopic methods, specific cultural tests, or in vitro or in vivo immunological tests.

§ 73.76¹ Requests for samples and protocols; official release.

Samples of any lot of any licensed product, together with the protocols showing results of applicable tests, may at any time be required to be sent to the Director, Division of Biologics Standards. Upon notification by the Director, Division of Biologics Standards, a manufacturer shall not distribute a lot of a product until the lot is released by the Director, Division of Biologics Standards: *Provided*, That the Director shall not issue such notification except when deemed necessary for the safety, purity or potency of the product.

§ 73.77¹ Cultures.

(a) *Storage and maintenance.* Cultures used in the manufacture of products shall be stored in a secure and orderly manner, at a temperature and by a method that will retain the initial characteristics of the organisms and insure freedom from contamination and deterioration.

(b) *Identity and verification.* Each culture shall be clearly identified as to source strain. A complete identification of the strain shall be made for each new stock culture preparation. Primary and subsequent seed lots shall be identified by lot number and date of preparation. Periodic tests shall be performed as often as necessary to verify the integrity of the strain characteristics and freedom from extraneous organisms. Results of all periodic tests for verification of cultures and determination of freedom from extraneous organisms shall be recorded and retained.

¹ Sec. 73.75 redesignated (formerly 73.72(b)) and amended; Sec. 73.76 redesignated (formerly 73.75); and a new Sec. 73.77 added June 28, 1961, 26 FR 5754, to become effective July 28, 1961.

§ 73.78¹ Ingredients, preservatives, diluents.

All ingredients used in a licensed product, and any diluent provided as an aid in the administration of the product shall meet generally accepted standards of purity and quality. Any preservative used shall be sufficiently non-toxic so that the amount present in the recommended dose of the product will not be toxic to the recipient, and in the combination used shall not denature the specific substances in the product below the minimum acceptable potency within the dating period when stored at the recommended temperature.

§ 73.79¹ Total solids in serums.

Except as otherwise provided by regulation, no liquid serum or antitoxin shall contain more than 20 percent total solids.

§ 73.80¹ Permissible combinations.

Licensed products may not be combined with other licensed products, either therapeutic, prophylactic or diagnostic, except as a license is obtained for the combined product. Licensed products may not be combined with non-licensable therapeutic, prophylactic or diagnostic substances except as a license is obtained for such combination.

§ 73.81¹ Standard preparations.

Standard preparations made available by the Director, Division of Biologics Standards, shall be applied in testing for potency all forms of Diphtheria Antitoxin; Tetanus Antitoxin; Botulism Antitoxin, Type A; Botulism Antitoxin, Type B; Histolyticus Antitoxin; Oedematiens Antitoxin; Perfringens Antitoxin; Sordelli Antitoxin; Vibrion Septique Antitoxin; Staphylococcus Antitoxin; Scarlet Fever Streptococcus Antitoxin; Dysentery Antitoxin (Shiga); Antipneumococcal Serum, (Types I, II, V, VII, and VIII); Anti-hemophilus Influenzae Type b Serum; Anti-rabies Serum; Pertussis Vaccine; Tuberculin, Old; and Tuberculin, Purified Protein Derivative.²

¹ Sec. 73.79 deleted; 73.78, 73.80, 73.81, 73.82, 73.83 redesignated as 73.80, 73.81, 73.82, 73.83 and 73.84 respectively; and redesignated 73.83 amended; June 28, 1961, 26 FR 5755, to become effective July 28, 1961.

² Secs. 73.81 and 73.82 amended 9/16/61, 26 FR 8668.

§ 73.82¹ Limits of potency.

Diphtheria Antitoxin shall have a potency of not less than 500 units per milliliter. Tetanus Antitoxin shall have a potency of not less than 400 units per milliliter. Scarlet Fever Streptococcus Antitoxin shall have a potency of not less than 400 units per milliliter. Pertussis Vaccine shall have a potency of 12 units per total human immunizing dose, based upon test values of not less than 8 units nor more than 36 units. Products dispensed in the dried state shall represent liquid products having these potency limitations.²

§ 73.83¹ Date of manufacture.

The dating period of a product kept at the manufacturer's recommended storage temperature, shall begin on the date of manufacture, which shall be determined as follows:

(a) For products for which an official standard of potency is prescribed in either § 73.81 or § 73.82, or which are subject to official potency tests, the date of initiation by the manufacturer of the last valid potency test.

(b) For products which are not subject to official potency tests, (1) the date of removal from animals, (2) the date of extraction, (3) the date of solution, or (4) the date of cessation of growth, whichever is applicable.

§ 73.84³ Periods of cold storage.

Except as otherwise provided in the regulations of this part, products may be held by the manufacturer in cold storage after the date of manufacture without decreasing the dating period, for the following periods:

At a temperature not above 10° C., for six months, or

At a temperature not above 5° C., for one year, or

At a temperature not above 0° C., for two years.

§ 73.85³ Expiration date; dating period.

The expiration date for each product shall be no later than the last day of the dating period. The dating period for a product shall begin either on the date of manufacture or on any date not later than the date of issue from the manufacturer.

³ Sec. 73.84 amended and Sec. 73.85 added 9/16/61, 26 FR 8668.

turer's cold storage, except that if stored for longer than the period prescribed in § 73.84, the dating period shall begin no later than the last day of the prescribed cold storage period.

ADDITIONAL STANDARDS: TRIVALENT ORGANIC
ARSENICALS

§ 73.90 Tests prior to release.

Tests required to be made, prior to the release of each lot of a licensed product, shall be supplemented in the case of the trivalent organic arsenicals by tests for:

- (a) Stability,
- (b) Solubility,
- (c) Arsenic content,
- (d) Moisture,
- (e) Relative non-toxicity.

§ 73.91 Pre-testing by Institutes; sample of each lot.

Prior to the release of any lot of the product, the manufacturer shall forward to the Director, Division of Biologics Standards, no less than 15 ampoules of the largest single-dose size in such lot, together with protocols showing the results of each test required prior to release.

§ 73.92 Expiration date.

Notification from the Director, Division of Biologics Standards, that lot samples forwarded in accordance with § 73.91 have satisfactorily passed prescribed tests shall indicate a date which may be taken as the date of manufacture for the purpose of fixing the expiration date. The date of issue shall be the same as the date of manufacture.

§ 73.93 Composition of product.

Solutions or solutions of mixtures in the concentrations recommended for clinical administration shall be of such hydrogen ion value and tonicity as to be physiologically compatible with human blood.

§ 73.94¹ Container.

The product shall be hermetically sealed under vacuum or under a dry non-oxidizing gas in glass ampoules. The contents of any final container shall not exceed 10 maximum human doses.

§ 73.95 Final container label.

In addition to the labeling requirements stated in § 73.50 the final container label of the trivalent organic arsenicals shall bear the statements required in § 73.96 (b) and (c) and an additional statement giving the amount of the drug contained in the ampoule.

§ 73.96 Outside label.

The outside label, in addition to the complete proper name and all other items required for products generally shall show conspicuously: (a) If the product is dispensed as a mixture or solution, the name of all admixed substances,

(b) If the ampoule is a multiple dose container, the fact that it is a multiple dose container,

(c) Specific method of preparation, if any, required prior to administration, as, for example, alkalization.

ADDITIONAL STANDARDS: POLIOMYELITIS VACCINE

§ 73.100 The product.

(a) *Proper name and definition.* For the purpose of section 351(a)(2) of the Act and § 73.1(k), the proper name of this product shall be "Polio-myelitis Vaccine", which shall consist of an aqueous preparation of poliomyelitis viruses types 1, 2, and 3, grown in monkey kidney tissue cultures, inactivated by a suitable method.

(b) *Strains of virus.* Strains of poliomyelitis virus used in the manufacture of vaccine shall be identified by historical records, infectivity tests and immunological methods. Any strain of virus may be used that produces a vaccine meeting the safety and potency requirements of §§ 73.101, 73.102, and 73.103, but the Surgeon General may from time to time prohibit the use of any specific

¹ Sec. 73.94 amended June 28, 1961, FR 5755, to become effective July 28, 1961.

strain whenever he finds that it is practicable to use another strain of the same type that is potentially less pathogenic to man and that will produce a vaccine of equivalent safety and potency.

(c) *Monkeys.* Only cynomolgus or rhesus monkeys, or other species of equal suitability, in overt good health that have reacted negatively to tuberculin within two weeks prior to use, shall be used as the source of kidney tissue for the production of virus. Each animal shall be examined at necropsy under the supervision of a qualified pathologist for gross signs of disease and, if there is any gross pathological lesion of significance to their use in the manufacture of the vaccine, the kidneys shall be discarded. Kidney tissue from monkeys that have been used previously for experimental purposes shall not be used, except that monkeys in overt good health, used for the safety or potency tests with negative clinical findings (§§ 73.102 and 73.103) that have reacted negatively to tuberculin prior to such test, may be used within two weeks of the end of the test period.

§ 73.101¹ Manufacture of poliomyelitis vaccine.

(a) *Cultivation of virus.* Virus for manufacturing vaccine shall be grown with aseptic techniques in monkey kidney cell tissue cultures. Suitable antibiotics in the minimum concentration required may be used. If penicillin is used, not more than 200 units per milliliter may be added.

(b) *Filtration.* Within 72 hours preceding the beginning of inactivation, the virus suspensions shall be filtered or clarified by a method having an efficiency equivalent to that of filtration through an S1 Seitz type filter pad.

(c) *Virus titer.* The 50 percent end-point (TCID₅₀) of the virus fluids after filtration shall be 10⁻⁶ or greater as confirmed by comparison in a simultaneous test (using groups of 10 tubes at 1 log steps or groups of 5 tubes at 0.5 log steps) with a reference virus distributed by the National Institutes of Health. Acceptable titrations of the reference virus shall not vary more than ±10-fold from its labeled titer using 0.5 milliliter inoculum in tissue culture.

(d)¹ *Inactivation of virus.* The virus shall be inactivated, as evidenced by the tests prescribed

in § 73.102, through the use of an agent or method which has been demonstrated to the satisfaction of the Surgeon General to be consistently effective in the hands of the manufacturer in inactivating a series of lots of poliomyelitis virus. If formaldehyde is used for inactivation, it shall be added to the virus suspension to a final concentration of U.S.P. solution of formaldehyde of 1:4000, and the inactivation conducted under controlled conditions of pH and time, at a temperature of 36° to 38° C. Three or more virus titers, suitably spaced to indicate rate of inactivation, shall be determined during the inactivation process. Filtration equivalent to that described in paragraph (b) of this section shall be performed after the estimated baseline time (time at which the 50 percent end-point reaches one tissue culture infective dose per milliliter), but prior to sampling for the first single strain tissue culture test required in § 73.102(b), except that this filtration may be omitted for strains of a virulence for monkeys equal to or less than that of the MEF-1 Type 2 strain of poliovirus.

(e) *Additional processing.* Single strain or trivalent pools that have failed to pass safety tests prescribed in § 73.102 (b), (c), or (e) may be treated as follows:

(1) Filtration or clarification by a method having an efficiency equivalent to that of filtration through an S1 Seitz type filter pad.

(2) Negative tests performed as described in § 73.102 (b) and (c) must be obtained on each of two successive samples taken so as to be separated by an interval of at least three days while the material is being subjected to treatment with 1:4000 U.S.P. formaldehyde solution and heat at 36° to 38° C. The first sample may be taken before incubation is begun and the second sample is to be taken after the incubation of at least 3 days is completed. In the case of single strain pools, the volume tested for each tissue culture safety test shall be at least 500 milliliters and in the case of trivalent pools, at least 1,500 milliliters.

(3) Pools which are positive following such additional processing shall not be used for the manufacture of poliomyelitis vaccine.

(f) *Supplemental inactivation.* Supplemental inactivation employing a method capable of reducing the titer of a similarly produced virus suspension by a factor of 10⁻⁶ may be applied at

¹ Sec. 73.101(d) amended August 4, 1960, 25 FR 7317.

any point after the filtration step described in paragraph (d) or (e)(1) of this section.

§ 73.102 Tests for safety.

In the manufacture of the product, the following tests relating to safety shall be conducted by the manufacturer.

(a)¹ *The virus pool—tests prior to inactivation*—(1) *B virus and Mycobacterium tuberculosis*. Prior to inactivation, each individual virus harvest or virus pool shall be tested for the presence of B virus and Mycobacterium tuberculosis.

(2) *SV₄₀*. Prior to inactivation, the material shall be tested for the presence of SV₄₀ as follows (or by any other test producing equally reliable results): A sample of at least five ml. from the virus harvest or virus pool or pool of tissue culture fluids from corresponding control vessels shall be neutralized by high titer antiserum of an origin other than human, chicken, or simian. The sample shall be tested in the same tissue culture system used for propagating the virus vaccine, and in primary cercopithecus tissue cultures or in a cell line demonstrated as equally susceptible to SV₄₀. Each tissue culture system shall be observed for at least 14 days and at the end of the observation period at least one subculture of fluid shall be made in the same tissue culture system and observed for an additional 14 days.

(3) *Test results*. The virus harvest or virus pool is satisfactory for poliomyelitis vaccine only if the tests produce no evidence of the presence of B virus, Mycobacterium tuberculosis or SV₄₀.

(b) *Single strain pool tissue culture tests for poliomyelitis virus*. (1) During inactivation, before pooling to make the final poliomyelitis vaccine, each monovalent bulk strain pool shall be tested for the presence of virus by tissue culture methods. Two samples separated by an interval of at least three days during inactivation at 36° to 38° C. shall be tested.

(2)² No less than 500 milliliters of each sample shall be subjected to the complete testing procedure and each test shall be performed on a different monkey kidney tissue culture cell preparation. Each sample shall be inoculated into five

or more tissue culture bottles of a suitable capacity, the ratio of the vaccine to the nutrient fluid being approximately 1:1 to 1:3, and the area of the surface growth of cells being at least 3 square centimeters per milliliter of vaccine. The tissue culture bottles shall be observed at least 14 days.

(3) A first subculture shall be made at the end of seven days from date of inoculation by planting at least 2 percent of the volume from each original bottle into suitable tissue culture containers, followed by refeeding.

(4) A second subculture shall be made from each original bottle in the same manner at the end of 14 days from date of inoculation.

(5) The first and second subcultures shall be each observed for at least seven days.

(6) If cytopathogenic effects occur either in the original bottles of the two tests or in the subcultures from them, or if cellular degeneration appears in the original bottles or in the subcultures before degeneration occurs in uninoculated cultures, the pool shall be held until the matter is resolved. If active poliomyelitis virus is indicated, the strain pool shall not be used for inclusion in a final vaccine unless effectively reprocessed as described in § 73.101(e). If other viruses are present, the pool shall not be used unless it can be demonstrated that such viruses have originated from other than the strain pool being tested.

(c) *Trivalent vaccine pool tissue culture test*. No less than 1,500 milliliters of the trivalent vaccine pool, without final preservative, prepared by pooling the three type pools, each of which has passed all tests described in paragraph (b) of this section, shall be subjected to the complete tissue culture test prescribed in such paragraph in at least two approximately equal tests in separate monkey kidney tissue culture preparations.

(d) *Trivalent vaccine pool lymphocytic choriomeningitis test*. The final vaccine shall be shown to be free of lymphocytic choriomeningitis virus by intracerebral inoculation into 10 or more mice which shall be observed daily for at least 21 days and a negative test shall not be valid unless at least 8 mice survive for this period.

(e) *Final vaccine test for active virus in monkeys*. (1) Vaccine from a sufficient number of final containers selected at random from each filling of each lot shall be pooled to provide a test

¹ Sec. 73.102(a) amended and effective 3/5/63, 28 FR 2110.

² Sec. 73.102(b)(2) amended 6/28/61, 26 FR 5755, effective 7/28/61.

sample of at least 100 milliliters representing the filling.

(2) Where the number of fillings in a lot is four or more, a test sample from each filling shall be inoculated into a group of five or more monkeys. Where the number of fillings in a lot is less than four, test samples from the several fillings shall be inoculated into a total of not less than twenty monkeys. A preinjection serum sample must contain no neutralizing antibody against the three poliomyelitis virus types in a dilution of 1:4 when tested against not more than 1,000 TCID₅₀ doses of virus. At least 80 percent of the test animals representing each filling must survive the test period without significant weight loss, except that if at least 60 percent of the test animals survive the first 48 hours after injection, those animals which do not survive this 48 hour period may be replaced by an equal number of test animals. If less than 60 percent of the test animals survive the first 48 hours, or if less than 80 percent of the animals representing each filling survive the test period without significant weight loss, the test must be repeated.

(3) Vaccine is injected by combined intracerebral, intraspinal, and intramuscular routes into rhesus or cynomolgus monkeys in overt good health, under deep barbiturate anesthesia. The intracerebral injection consists of 0.5 milliliter into the thalamic region of each hemisphere. The intraspinal injection consists of 0.5 milliliter into the lumbar spinal cord enlargement. The intramuscular injection consists of 1.0 milliliter into the right leg muscles. At the same time, an injection of 200 milligrams of cortisone acetate is given into the left leg muscles, and 1.0 milliliter of procaine penicillin (300,000 units) into the right arm muscles. The monkeys are observed for 17 to 19 days and symptoms suggestive of poliomyelitis are recorded.

(4) At the end of the observation period, samples of nervous tissue are taken for virus recovery and identification. Histological sections are prepared from both spinal cord enlargements and examined.

(5) Doubtful histopathological findings necessitate (i) examination of a sample of sections from several regions of the brain in question, and (ii) attempts at virus recovery from the nervous tis-

sues previously removed from the animal. The test is considered negative if the histological and other studies leave no doubt that poliomyelitis infection did not occur.

§ 73.103 Potency test.

Each lot of vaccine shall be subject to a potency test which permits an estimation of the antigenic capacity of the vaccine. This is done by means of a simultaneous comparison of the antibody levels produced in monkeys by the vaccine under test with the antibody levels of reference serums distributed by the National Institutes of Health. The potency test shall be performed on samples taken after final processing of the trivalent pool including addition of preservative, and shall be conducted as follows:

(a) *Inoculation of monkeys.* A group of 12 or more rhesus or cynomolgus monkeys shall be used. Animals shall weigh between 4 and 8 pounds and shall be in overt good health. Animals that become ill and then remain ill during the course of immunization shall be excluded from the group. The test shall not be valid unless at least 8 survive the test period. The test vaccine shall be given intramuscularly to each monkey in 3 doses of 1.0 milliliter each at seven day intervals. Only undiluted vaccine shall be used.

(b) *Serum samples.* A blood sample shall be taken from each monkey prior to vaccination and then again 7 days after the last injection. Serum shall be separated aseptically, and stored under refrigeration.

(c) *Serum-virus neutralization test.* The titers of individual monkey serums shall be determined in comparison with NIH reference serums in tests designed to include controls for all the variables of significance including the following:

- (1) Serum toxicity control;
- (2) Cell control and cell titration;
- (3) Virus titration control (at least 4 tubes/dilution at 1/2 logs steps); and
- (4) Serum controls using type-specific serums to identify the type of virus used in the neutralization test.

(d) *Interpretation of the test.* Animals showing preinoculation titers of 1/4 or over when tested against not more than 1,000 TCID₅₀ of virus, shall

be excluded from the test. The geometric mean titer of antibody induced in the monkeys surviving the course of immunization and bleeding, shall be calculated. A comparison of the value so obtained shall be made with the values for the reference serums that were tested simultaneously and expressed as ratios between the mean titer values of the serums under test and the mean titer value of the reference serums.

(e) *Potency requirements.* A lot of vaccine tested against National Institutes of Health reference serums IIA, IIA $\frac{1}{4}$, and IIA $\frac{1}{16}$ shall be satisfactory if the geometric mean value of the group of individual monkey serums representing the lot of vaccine tested is at least 0.86 times the geometric mean value of the three reference serums for type 1, at least 0.75 times for type 2, and at least 0.48 times for type 3. In applying the foregoing requirements, a variation of 66 $\frac{2}{3}$ percent is acceptable.

§ 73.104 General requirements.

(a) ¹ *Final container tests.* Tests shall be made on final containers for safety, sterility and identity in accordance with §§ 73.72, 73.73 and 73.75 respectively. In addition, no lot of final vaccine shall be released unless it is one of a series of five consecutive lots produced by the same manufacturing process, all of which have shown negative results with respect to all tests for the presence of live poliovirus, and unless each of the monovalent pools of which a polyvalent final vaccine is composed similarly is one of a series of five consecutive monovalent pools of the same type of inactivated poliovirus, all of which have shown negative results in all tests for the presence of live poliovirus.

(b) *Extraneous protein.* Extraneous protein, capable of producing allergenic effects on injection into human subjects, shall not be added to the final virus production medium. If animal serum is used at any stage, its calculated concentration in the final medium shall not exceed 1:1,000,000.

(c) *Dose.* These additional standards are based on a human dose of 1.0 milliliter for a single injection and a total human immunizing dose of three injections of 1.0 milliliter given at appropriate intervals.

(d) *Labeling.* In addition to compliance with the requirements of §§ 73.50 to 73.55, inclusive, the label or the package enclosure shall include an appropriate statement indicating the type and amounts of antibiotics added, if any. The preservative used shall be stated on the label, as well as allergenic substances added, if any, and the source, composition and method of inactivation of the viruses.

(e) ¹ *Dating.* The expiration date shall be no more than six months after either the date of manufacture as defined in § 73.83(a) or the date of issue from cold storage. Such date of issue shall not be more than six months after such date of manufacture, and the product prior to issue shall be kept constantly at a temperature not exceeding 10°C.

(f) *Requirements for samples and reports.* For each lot of vaccine, the following material shall be submitted to the Director, Division of Biologics Standards, National Institutes of Health, Bethesda, Maryland 20014:

(1) A 2,500 milliliter sample, neutralized, not dialyzed, and without final preservative, taken at the latest possible stage of manufacturing before the addition of such preservative.

(2) A 200 milliliter bulk sample of the final vaccine containing final preservative.

(3) A total of not less than a 200 milliliter sample of the final vaccine in final labeled containers.

(4) Protocols showing the history of the lot and the results of the tests prescribed in these additional standards.

§ 73.105¹ Equivalent methods.

Modification of any particular manufacturing method or procedure or the conditions under which it is conducted as set forth in the additional standards relating to poliomyelitis vaccine (§§ 73.100 to 73.104, inclusive) shall be permitted whenever the manufacturer presents evidence to demonstrate that such modification will provide equal or greater assurances of the safety, purity and potency of the vaccine as the assurances provided by such standards, and the Surgeon General so finds and makes such finding a matter of official record.

(Sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216. Interprets or applies sec. 351, 58 Stat. 702; 42 U.S.C. 262)

¹ Secs. 73.104 (a) and (e), and 73.105 amended June 28, 1961, 26 FR 5755, to become effective July 28, 1961.

ADDITIONAL STANDARDS: POLIOVIRUS VACCINE,
LIVE, ORAL

§ 73.110¹ The product.

(a) *Proper name and definition.* For the purpose of section 351(a)(2) of the Act and § 73.1(k), the proper name of this product shall be "Poliovirus Vaccine, Live, Oral", followed by a designation of the form in which the vaccine is distributed by the manufacturer. The vaccine shall be a preparation of one or more live, attenuated polioviruses grown in monkey kidney cell cultures, prepared in a form suitable for oral administration.

(b) *Criteria for acceptable strains and acceptable seed virus.* (1) Strains of attenuated poliovirus Types 1, 2, and 3 used in the manufacture of the vaccine shall be identified by: (i) Historical records including origin and techniques of attenuation, (ii) antigenic properties, (iii) neurovirulence for monkeys, (iv) pathogenicity for other animals and tissue cultures of various cell types, and (v) established virus markers including ret/40, d, MS.

(2) Poliovirus strains shall not be used in the manufacture of Poliovirus Vaccine, Live, Oral, unless, (i) data are submitted to the Surgeon General which establish that each such strain is free of harmful effect upon administration in the recommended dosage to at least 100,000 people susceptible to poliomyelitis, under circumstances where adequate epidemiological surveillance of neurological illness has been maintained, and, (ii) each such strain produces a vaccine meeting the safety and potency requirements of §§ 73.114(b), 73.115, and 73.117. Susceptibility shall be demonstrated by blood tests, stool examinations and other appropriate methods.

(3) Each seed virus used in manufacture shall be demonstrated to be free of extraneous microbial agents.

(4) No seed virus shall be used for the manufacture of poliovirus vaccine unless its neurovirulence in *Macaca* monkeys is no greater than that of the NIH Reference Attenuated Poliovirus. The neurovirulence of the seed virus shall be dem-

onstrated by the following tests to be performed by the manufacturer: (i) The test prescribed in § 73.114(b)(1) using seed virus as test material in place of monovalent virus pool material and (ii) the following comparative intramuscular neurovirulence test: Each of at least ten monkeys shall be injected with a total of 5.0 ml. of the seed virus under test in one or more proximate locations of either a gluteus or gastrocnemius muscle. Similar injections shall be made in another group of ten monkeys using the NIH Reference Attenuated Poliovirus. Each monkey shall be injected intramuscularly with no less than $10^{7.7}$ TCID₅₀ of viral inoculum. All monkeys shall be observed for 17 to 21 days and a comparative evaluation shall be made of the evidence of neurovirulence of the virus under test and the NIH Reference Attenuated Poliovirus, as prescribed in § 73.114(b)(1)(iii).

(5) Subsequent and identical neurovirulence tests shall be performed in monkeys whenever there is evidence of a change in the neurovirulence of the production virus and upon introduction of a new production seed lot and as often as necessary otherwise to establish to the satisfaction of the Surgeon General that the seed virus strains for vaccine manufacture have maintained their neurovirulence properties as set forth in § 73.114(b)(1).

(6) The Surgeon General may, from time to time, prohibit the use of a specified strain whenever he finds it is practicable to use another strain of the same type which is potentially less pathogenic for man, and that it will produce a vaccine of equivalent safety and potency.

§ 73.111 NIH reference strains.

The following NIH reference viruses shall be obtained from the Division of Biological Standards.

NIH Reference Poliovirus, Live Attenuated, Type 1, as a control for correlation of virus titers in tissue cultures.

NIH Reference Poliovirus, Live Attenuated, Type 2, as a control for correlation of virus titers in tissue cultures.

NIH Reference Poliovirus, Live Attenuated, Type 3, as a control for correlation of virus titers in tissue cultures.

NIH Reference Attenuated Poliovirus, Type 1, as a control for correlation of monkey neurovirulence tests.

¹ Secs. 73.110 through 73.115 amended and Secs. 73.116 through 73.118 are added March 25, 1961, to become effective April 24, 1961, 25 FR 2565.

§ 73.112 Animal conditioning, personnel, and facilities.

(a) *Monkey conditioning, housing and handling.*

(1) Only *Macaca* monkeys, or a species found by the Director, Division of Biologics Standards, to be equally suitable, in overt good health, that have reacted negatively to tuberculin at the start of the prescribed quarantine period, shall be used as the source of kidney tissue for the manufacture of poliovirus vaccine.

(2) Monkeys that have been used previously for experimental purposes shall not be used as a source of kidney tissue in the manufacture of vaccine.

(3) Monkeys to be used as a source of kidney tissue in vaccine manufacture shall be maintained in quarantine for at least six weeks prior to use in cages closed on all sides with solid materials except the front, which shall be screened. Not more than two monkeys shall be housed in one cage, and cage mates shall not be interchanged.

(4) Excluding deaths from accidents or causes not due to infectious diseases, if the death rate of any group of monkeys being conditioned in accordance with subparagraph (3) of this paragraph exceeds five percent per month, the remaining monkeys may be used for poliovirus vaccine only if they survive a new quarantine period.

(5) Each animal at necropsy shall be examined under the direction of a qualified pathologist, physician, or veterinarian having experience with diseases of monkeys, for the presence of signs or symptoms of ill health, particularly for (i) evidence of tuberculosis, (ii) presence of herpes-like lesions, including eruptions or plaques on or around the lips, in the buccal cavity or on the gums and (iii) signs of conjunctivitis. If there are any such signs or other significant gross pathological lesions, the kidneys shall not be used in the manufacture of vaccine.

(b) *Personnel.* All possible steps shall be taken to insure that personnel involved in processing the vaccine are immune to poliovirus in order to minimize the possibility that they may become excretors of poliovirus.

(c) *Facilities.* The space set aside for work with the live poliovirus vaccine shall not be used for any other purpose during the vaccine processing period. All areas used for live poliovirus vaccine processing shall be decontaminated prior to

the initiation of such processing. Test procedures which potentially involve the presence of microorganisms including viruses other than the vaccine strains, or the use of tissue culture cell lines other than primary cultures, shall not be conducted in live poliovirus vaccine manufacturing areas.

§ 73.113 Manufacture of poliovirus vaccine, live, oral.

(a) *Primary cell cultures.* Only primary monkey kidney tissue cultures may be used in the manufacture of poliovirus vaccine. Continuous line cells shall not be introduced or propagated in vaccine manufacturing areas.

(b) *Virus passages.* Virus in the final product shall represent no more than five tissue culture passages from the original strain, each of which shall have met the criteria of acceptability prescribed in § 73.110(b).

(c) *Identification of processed kidneys.* The kidneys from each monkey shall be processed and the viral fluid resulting therefrom shall be identified as a separate monovalent harvest and kept separately from other monovalent harvests until all samples for the tests prescribed in the following paragraph relating to that pair of kidneys shall have been withdrawn from the harvest.

(d) *Monkey kidney tissue production vessels prior to virus inoculation.* Prior to inoculation with the seed virus, the tissue culture growth in vessels representing each pair of kidneys shall be examined microscopically for evidence of cell degeneration at least three days after complete formation of the tissue sheet. If such evidence is observed, the tissue from that pair of kidneys shall not be used for poliovirus vaccine manufacture. To test the tissue found free of cell degeneration for further evidence of freedom from demonstrable viable microbial agents, the fluid shall be removed from the cell cultures immediately prior to virus inoculation and tested in each of four culture systems; (1) *Macaca* monkey kidney cells, (2) *Cercopithecus* monkey kidney cells, (3) primary rabbit kidney cells, and (4) human cells (from one of the systems described in § 73.114(a)(6)), in the following manner: Aliquots of fluid from each vessel shall be pooled and at least ten ml. of the pool inoculated into each system, with ratios of inoculum to medium being 1:1 to 1:3

and with the area of surface growth of cells at least three square centimeters per milliliter of test inoculum. The cultures shall be observed for at least 14 days. If these tests indicate the presence in the tissue culture preparation of any viable microbial agent the tissue cultures so implicated shall not be used for poliovirus vaccine manufacture.

(e) *Control vessels.* Before inoculation with seed virus, sufficient tissue culture vessels to represent at least 25 percent of the cell suspension from each pair of kidneys shall be set aside as controls. The control vessels shall be examined microscopically for cell degeneration for an additional 14 days. The cell fluids from such control vessels shall be tested, both at the time of virus harvest and at the end of the additional observation period, by the same method prescribed for testing of fluids in the preceding paragraph (d) of this section. In addition the cell sheet in each control vessel shall be examined for presence of hemadsorption viruses by the addition of guinea pig red blood cells.

(f) *Virus harvest; interpretation of test results.* At least 80 percent of the control vessels shall successfully complete the additional 14-day observation period without microscopic evidence of cell degeneration of the tissue sheets. If less than 80 percent of the control vessels fail to complete satisfactorily the observation period, no tissue from the kidneys implicated shall be used for poliovirus vaccine manufacture. If the test results of the control vessels indicate the presence of any extraneous agent at the time of virus harvest, the entire virus harvest from that tissue culture preparation shall not be used for poliovirus vaccine manufacture. If any of the tests or observations described in paragraph (d) or (e) of this section demonstrate the presence in the tissue culture preparation of any microbial agent known to be capable of producing human disease, the virus grown in such tissue culture preparation shall not be used for poliovirus vaccine manufacture.

(g) *Kidney tissue production vessels after virus inoculation—temperature.* After virus inoculation, production vessels shall be maintained at a temperature not to exceed 35.0° C. during the course of virus propagation.

(h) *Kidney tissue virus harvests.* Virus harvested from vessels containing the kidney tissue from one monkey may constitute a monovalent virus pool and be tested separately, or viral har-

vests from more than one pair of kidneys may be combined, identified and tested as a monovalent pool. Each pool shall be mixed thoroughly and samples withdrawn for testing as prescribed in § 73.114(a). The samples shall be withdrawn immediately after harvesting and prior to further processing, except that samples of test materials frozen immediately after harvesting and maintained at -60° C. or below, may be tested upon thawing, provided no more than one freeze-thaw cycle is employed.

(i) *Filtration.* After harvesting and removal of samples for the safety tests described in § 73.114(a), the pool shall be passed through sterile filters having a sufficiently small porosity to assure bacteriologically sterile filtrates.

§ 73.114 Test for safety.

(a) *Tests prior to filtration.* Monovalent virus pools shall contain no demonstrable viable microbial agent other than the attenuated live polioviruses intended. The vaccine shall be tested for the absence of adventitious and other infectious agents including polioviruses of other types or strains, simian agents, *Mycobacterium tuberculosis*, pox viruses, lymphocytic choriomeningitis virus, Echo viruses, Coxsackie viruses, and B-virus. Testing of each monovalent pool shall include the following procedures:

(1) *Inoculation of rabbits.* A minimum of 100 ml. of each monovalent virus pool shall be tested by inoculation into at least ten healthy rabbits, each weighing 1500-2500 grams. Each rabbit shall be injected intradermally in multiple sites, with a total of 1.0 ml. and subcutaneously with 9.0 ml., of the viral pool, and the animals observed for at least three weeks. Each rabbit that dies after the first 24 hours of the test or is sacrificed because of illness shall be necropsied and the brain and organs removed and examined. The virus pool may be used for poliovirus vaccine only if at least 80 percent of the rabbits remain healthy and survive the entire period and if all the rabbits used in the test fail to show lesions of any kind at the sites of inoculation and fail to show evidence of B-virus or any other viral infection.

(2) *Inoculation of adult mice.* Each of at least 20 adult mice each weighing 15-20 grams shall be inoculated intraperitoneally with 0.5 ml. and

intracerebrally with 0.03 ml. of each monovalent virus pool to be tested. The mice shall be observed for 21 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection by direct observation and subinoculation of appropriate tissue into at least 5 additional mice which shall be observed for 21 days. The monovalent virus pool may be used for poliovirus vaccine only if at least 80 percent of the mice remain healthy and survive the entire period and if all the mice used in the test fail to show evidence of lymphocytic choriomeningitis virus or other viral infection.

(3) *Inoculation of suckling mice.* Each of at least 20 suckling mice less than 24 hours old, shall be inoculated intracerebrally with 0.01 ml. and intraperitoneally with 0.1 ml. of the monovalent virus pool to be tested. The mice shall be observed daily for at least 14 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness shall be necropsied and all areas examined for evidence of viral infection. Such examination shall include subinoculation of appropriate tissue suspensions into an additional group of at least five suckling mice by the intracerebral and intraperitoneal routes and daily observed for 14 days. In addition, a blind passage shall be made of a single pool of the emulsified tissue (minus skin and viscera) of all mice surviving the original 14 day test. The virus pool under test is satisfactory for poliovirus vaccine only if at least 80 percent of the mice remain healthy and survive the entire period and if all the mice used in the test fail to show evidence of Cocksackie or other viral infection.

(4) *Inoculation of guinea pigs.* Each of at least five guinea pigs, each weighing 350-450 grams, shall be inoculated intracerebrally with 0.1 ml. and intraperitoneally with 5.0 ml. of the monovalent virus pool to be tested. The animals shall be observed for at least 42 days and daily rectal temperatures recorded for the last three weeks of the test. Each animal that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied. The tissues shall be examined both microscopically and culturally for evidence of tubercle bacilli, and by passage of tissue suspensions into at least three other guinea pigs by the intracerebral and intraperitoneal

routes of inoculation for evidence of viral infection. If clinical symptoms suggest infection with lymphocytic choriomeningitis virus, serological tests shall be performed on blood samples of the test guinea pigs to confirm the clinical observations. Animals that die or are sacrificed during the first three weeks after inoculation with poliovirus shall be examined for infection with lymphocytic choriomeningitis virus. Animals that die in the final three weeks shall be examined both microscopically and culturally for *M. Tuberculosis*. The monovalent virus pool is satisfactory for poliovirus vaccine only if at least 80 percent of all animals remain healthy and survive the observation period and if all the animals used in the test fail to show evidence of infection with *M. Tuberculosis*, or any viral infection.

(5) *Inoculation of monkey kidney tissue cultures.* At least 500 doses or 50 ml., whichever represents a greater volume of virus, of each undiluted monovalent virus pool or in equal proportions from individual harvests or sub-pools, shall be tested for simian viruses in *Macaca*, and the same volume in *Cercopithecus*, monkey kidney tissue culture preparations, in a ratio of inoculum to medium of from 1:1 to 1:3, and with the area of surface growth of cells at least three square centimeters per milliliter of test inoculum, after neutralization of the poliovirus by high titer type specific non-simian antisera. The immunizing antigens used for the preparation of antisera shall be grown in a human tissue culture cell line. The cultures shall be observed for no less than 14 days. The monovalent virus pool is satisfactory for poliovirus vaccine only if all the tissue cultures fail to show evidence of the presence of simian viruses.

(6) *Inoculation of human cell cultures.* At least 500 doses or 50 ml., whichever represents a greater volume of virus, taken from either a single monovalent pool or in equal proportions from individual harvests or sub-pools, shall be tested in a ratio of inoculum to medium of 1:1 to 1:3, and with the area of surface growth of cells at least three square centimeters per milliliter of test inoculum, for the presence of measles virus in either (i) primary human amnion cells, (ii) primary human kidney cells, or (iii) any other cell system of comparable susceptibility to unmodified measles virus. The test material shall be neutralized with polio-

virus antiserum of non-simian derivation if the tissue culture cell system used is susceptible to poliovirus. The culture shall be observed for no less than 14 days. The monovalent virus pool is satisfactory for poliovirus vaccine only if all tissue cultures fail to show evidence of the presence of measles virus.

(7) *Inoculation of rabbit kidney tissue cultures.* At least 500 ml. of virus pool taken from either a single monovalent pool or in equal proportions from individual harvests or sub-pools, shall be tested in a ratio of inoculum to medium of from 1:1 to 1:3, and with the area of surface growth of cells at least three square centimeters per milliliter of test inoculum, in primary rabbit kidney tissue culture preparations for evidence of B-virus. The culture shall be observed for no less than 14 days. The monovalent virus pool is satisfactory for poliovirus vaccine only if all tissue cultures fail to show evidence of the presence of B-virus.

(b) *Tests after filtration.* The following tests relating to safety shall be performed after the filtration process, on each monovalent virus pool or on each multiple thereof (monovalent lot):

(1) *Neurovirulence in monkeys.* Each monovalent virus pool or monovalent lot shall be tested in comparison with the NIH Reference Attenuated Poliovirus for neurovirulence in Macaca monkeys by both the intrathalamic and intraspinal routes of injection. A preinjection serum sample obtained from each monkey must be shown to contain no neutralizing antibody in a dilution of 1:4 when tested against no more than 1,000 TCID₅₀ of each of the three types of poliovirus. The neurovirulence tests are not valid unless the sample contains at least 10^{7.0} TCID₅₀ per ml. when titrated in comparison with the NIH Reference Poliovirus, Live, Attenuated of the appropriate type. All monkeys shall be observed for 17 to 21 days, under the supervision of a qualified pathologist, physician or veterinarian, and any evidence of physical abnormalities indicative of poliomyelitis or other viral infections shall be recorded.

(i) *Intrathalamic inoculation.* Each of at least ten Macaca monkeys shall be injected intrathalamically with 1.0 ml. of virus pool material containing at least 10^{7.0} TCID₅₀ per ml. and each of at least ten additional Macaca monkeys shall be injected intrathalamically with 1.0 ml. of a 10⁻¹

dilution thereof. Comparative evaluations shall be made with the virus pool under test and the NIH reference material. Only monkeys that show evidence of inoculation into the thalamus shall be considered as having been injected satisfactorily.

(ii) *Intraspinal inoculation.* Each of a group of at least five Macaca monkeys shall be injected intraspinally with 0.2 ml. of virus pool material containing at least 10^{7.0} TCID₅₀ per ml. and each monkey in four additional groups of at least five Macaca monkeys shall be injected intraspinally with 0.2 ml. of 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴ dilutions thereof, respectively. Comparative evaluations shall be made with the virus pool under test and the NIH reference material. Only monkeys that show microscopic evidence of inoculation into the gray matter of the lumbar cord shall be considered as having been injected satisfactorily.

(iii) *Determination of neurovirulence.* At the conclusion of the observation period comparative histopathological examinations shall be made of the lumbar cord, cervical cord, lower medulla, upper medulla and mesencephalon of each monkey in the groups injected with virus under test and those injected with the NIH Reference Attenuated poliovirus, except that for animals dying during the test period, these examinations shall be made immediately after death. The animals shall be examined to ascertain whether the distribution and histological nature of the lesions are characteristic of poliovirus infection. A comparative evaluation shall be made of the evidence of neurovirulence of the virus under test and the NIH Reference Attenuated Poliovirus with respect to (a) the number of animals showing lesions characteristic of poliovirus infection, (b) the number of animals showing lesions other than those characteristic of poliovirus infection, (c) the severity of the lesions, (d) the degree of dissemination of the lesions, and (e) the rate of occurrence of paralysis not attributable to the mechanical injury resulting from inoculation trauma. The virus pool under test is satisfactory for poliovirus vaccine manufacture only if at least 80 percent of the animals in each group survive the observation period and if a comparative analysis of the test results demonstrate that the neurovirulence of the test virus pool does not exceed that of the NIH Reference Attenuated Poliovirus.

(2) *Test for virus titer.* The concentration of living virus in each monovalent virus pool or lot shall be determined, using the NIH Reference Poliovirus Live, Attenuated of the same type as a control. The test shall be a 50 percent end-point titration calculation ($TCID_{50}$), performed with either groups of ten tubes at 1 log dilution steps or groups of five tubes of 0.5 log dilution steps, or a test of demonstrated equivalent sensitivity. Acceptable titrations of the reference virus shall not vary more than ± 0.5 log from its labeled titer.

(3) *Tests for In-vitro Markers.* A test shall be performed on each monovalent virus pool or each monovalent lot resulting therefrom, using the rct/40 Marker and at least one of the other marker methods described below. The test results shall demonstrate that the virus under test and the seed virus have substantially the same marker characteristics.

(i) *rct/40 Marker.* Attenuated strains which grow readily at 40° C. ($\pm 0.5^{\circ}$ C.) are classified as rct/40 positive (+) in contrast to the rct/40 negative (-) strains which show an increased growth of at least 100,000 fold at 36° C. over that obtained at 40° C. Comparative determinations shall be made in either tube or bottle cultures.

(ii) *d Marker.* Attenuated strains which grow readily at low concentrations of bicarbonate under agar are classified as d positive (+) in contrast to the d negative (-) strains which exhibit delayed growth under the same conditions. The cultures shall be grown in a 36° C. incubator either in stoppered bottles or in plates in an environment of 5 percent CO_2 in air.

(iii) *MS Markers.* Attenuated strains which grow more readily on monkey stable (MS) cells are classified as MS positive (+) in contrast to the MS negative (-) strains. Comparative determinations shall be made in either tube cultures or in bottle cultures under agar.

(4) *Test for sterility.* The final bulk vaccine of each monovalent virus pool, or each monovalent lot resulting therefrom, shall be tested for sterility in both fluid Thioglycollate and Sabouraud media by the procedure prescribed in § 73.73 with a sample of no less than 10 ml. for each test.

§ 73.115 Potency test.

The concentration of live virus expressed as $TCID_{50}$ of each type in the vaccine shall constitute

the measure of its potency. The accuracy of the titration to determine the concentration of live virus in the lot under test shall be confirmed by performing a titration with the NIH Reference Poliovirus, Live, Attenuated of the appropriate type as a check on titration technique. The concentration of each type of live virus contained in the vaccine of the lot under test shall be between 200,000 and 500,000 $TCID_{50}$ per human dose.

§ 73.116 General requirements.

(a) *Repeat tests.* Tests may be repeated when it is demonstrated that the results were due to faulty test techniques.

(b) *Final container tests.* Tests shall be made on final containers for identity and safety in accordance with § 73.72. The final container sterility test need not be performed provided aseptic techniques are used in the filling process.

(c) *Consistency of manufacture.* No lot of vaccine shall be released unless each monovalent pool contained therein is one of a series of five consecutive pools of the same type, each pool having been manufactured by the same procedures, and each having met the criteria of neurovirulence for monkey keys prescribed in § 73.114(b) (1), and of in-vitro markers as prescribed in § 73.114(b) (3).

(d) *Dose.* The individual human dose of vaccine shall contain from 200,000 to 500,000 $TCID_{50}$ of each type of virus that is in the final product.

(e) *Labeling.* Labeling shall comply with the requirements of §§ 73.50 to 73.55, inclusive. In addition the label or a package enclosure shall include the identification and source of the virus or viruses contained in the vaccine, the tissue medium on which the virus or viruses were propagated, stabilizers and preservatives if any, and the type and calculated maximum amount of antibiotics.

(f) *Dating.* (1) The expiration date in no event shall be more than two years after the date of manufacture as defined in § 73.82(a) provided the product is maintained in the frozen state, (2) the expiration date shall be no more than one year from the date of issue provided that the product is maintained in the frozen state, and (3) the expiration date shall be no more than seven days from the date of issue if issued as a liquid and provided it is maintained at a temperature no higher than 10° C.

(g) *Samples and reports.* For each lot of vaccine, the following materials shall be submitted to the Director, Division of Biologics Standards, National Institutes of Health, Bethesda, Maryland 20014:

(1) All protocols relating to the history of manufacture of each lot of vaccine, and the results of all tests performed.

(2) A one liter bulk sample of each final monovalent pool having a virus titer of no less than $10^{7.5}$ TCID₅₀ per milliliter, except that if the titer is greater, a correspondingly smaller volume may be submitted.

(3) A total of no less than a 200 milliliter sample of the vaccine in final labeled containers.

§ 73.117 Clinical trials to qualify for license.

To qualify for license, the antigenicity of the vaccine shall have been determined by clinical trials of adequate statistical design, by oral administration of the product. Such clinical trials shall be conducted with five consecutive lots of poliovirus vaccine which have been manufactured by the same methods, each of which has shown satisfactory results in all prescribed tests. Type specific neutralizing antibody (from less than 1:4 before vaccine treatment, to 1:16 or greater after treatment) shall be induced in 80 percent or more of susceptibles when administered orally as a single dose or in excess of 90 percent of susceptibles when administered orally after a series of doses. A separate clinical trial shall have been conducted for each monovalent and each polyvalent vaccine for which license application is made.

§ 73.118 Equivalent methods.

Modification of any particular manufacturing method or process or the conditions under which it is conducted as set forth in the additional standards relating to Poliovirus Vaccine, Live, Oral, shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide assurances of the safety, purity, and potency of the vaccine that are equal to or greater than the assurances provided by such standards, and the Surgeon General so finds and makes such finding a matter of official record.

(Sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216. Interpret or apply Sec. 351, 58 Stat. 702, as amended; 42 U.S.C. 262)

ADDITIONAL STANDARDS: ADENOVIRUS VACCINE

§ 73.130 The product.¹

(a) *Proper name and definition.* For the purpose of section 351(a)(2) of the Act and § 73.1(k), the proper name of this product shall be "Adenovirus Vaccine" with a designation of the types of virus included in the vaccine. Such vaccine shall consist of an aqueous preparation of one or more adenoviruses grown in monkey kidney tissue cultures inactivated by a suitable method. Where more than one type of virus is used in the manufacture of the vaccine, equal proportions of each type shall be combined with a tolerance for each component of 5 percent of the total volume.

(b) *Strains of virus.* Strains of adenovirus used in the manufacture of the vaccine shall be identified by historical records, infectivity tests, and immunological methods. Only those strains of virus may be used that (1) produce a vaccine meeting the safety and potency requirements in §§ 73.132 and 73.133, (2) never had any passage in malignant cells of human or animal origin, and (3) have been maintained in monkey kidney cultures for at least 10 passages prior to use.

(c) *Monkey kidney tissue.* Only cynomolgus or rhesus monkeys or other species of equal suitability, in overt good health, that have reacted negatively to tuberculin within 2 weeks prior to use shall be used as a source of kidney tissue for the production of virus. Each animal shall be examined at necropsy under the supervision of a qualified pathologist for gross signs of disease. If there is any gross pathological lesion of any significance to their use in the manufacture of vaccine, the kidneys shall be discarded. Kidney tissue from monkeys that have been used previously for experimental purposes shall not be used, except that monkeys in overt good health, used for the safety or potency tests of adenovirus vaccines with negative clinical findings (§§ 73.132 and 73.133) that have reacted negatively to tuber-

¹ Secs. 73.130 through 73.135 (formerly secs. 73.110 through 73.115) redesignated as such March 25, 1961, to become effective April 24, 1961, 26 FR 2565.

culin prior to such test, may be used within two weeks of the end of the test period. The monkeys shall not at any time have been housed in the same building where monkeys actually infected with or exposed to poliovirus are housed, and due precautions shall be taken to prevent cross-infection from any infected or potentially infected monkeys on the premises.

§ 73.131 Manufacture of adenovirus vaccine.

(a) *Cultivation of virus.* Virus for manufacturing vaccine shall be grown with aseptic technique in monkey kidney cell cultures using a synthetic medium. Suitable antibiotics in the minimum concentration required may be used. If penicillin is used, not more than 200 units per milliliter may be added. Phenol red may not exceed a concentration of 0.002 percent.

(b) *Filtration.* Within 72 hours preceding the beginning of inactivation, the virus suspensions shall be filtered or clarified by a method having an efficiency at least equivalent to that of a Selas 02 type filter.

(c) *Virus titer.* The titer of each virus pool after filtration shall be determined by a suitable method. It shall also be demonstrated that each virus pool possesses adenovirus group antigen by the complement-fixing test.

(d) *Inactivation of virus.* The virus shall be inactivated, as evidenced by the test in tissue culture as set forth in § 73.132, through the use of an agent or method which has been demonstrated to be effective in the hands of the manufacturer in inactivating a series of at least 5 consecutive lots of adenovirus vaccine. If formaldehyde is used for inactivation, it shall be added to the virus suspension to a final concentration of U.S.P. formaldehyde solution of at least 1:4000. The inactivation shall be conducted under controlled conditions of pH and time at a temperature of 36° to 38° C. As an indication of inactivation, not less than two samples shall be removed during the inactivation process and treated as prescribed in § 73.132(b) (1). Regardless of the concentration of formaldehyde used, the total heating period shall be not less than 20 hours and at least three times the period required for the reduction of live virus to a point where no virus is detected in a 5 milliliter sample when tested in accordance with § 73.132

(b) (1). At the end of the heating period a sample shall be removed for the single strain tissue culture safety test.

§ 73.132 Tests for safety.

In the manufacture of the product, the following tests relating to safety shall be conducted by the manufacturer:

(a) *The virus pool*—(1) ¹ *Tests prior to inactivation*—(i) *B virus and Mycobacterium tuberculosis.* Prior to inactivation, each individual virus harvest or virus pool shall be tested for the presence of B virus and Mycobacterium tuberculosis.

(ii) *SV₄₀.* Prior to inactivation, the material shall be tested for the presence of SV₄₀ as follows (or by any other test producing equally reliable results): A sample of at least five ml. from the virus harvest or virus pool or pool of tissue culture fluids from corresponding control vessels shall be neutralized by high titer antiserum of an origin other than human, chicken, or simian. The sample shall be tested in the same tissue culture system used for propagating the virus vaccine, and in primary cercopithecus tissue cultures or in a cell line demonstrated as equally susceptible to SV₄₀. Each tissue culture system shall be observed for at least 14 days and at the end of the observation period at least one subculture of fluid shall be made in the same tissue culture system and observed for an additional 14 days.

(iii) *Test results.* The virus harvest or virus pool is satisfactory for adenovirus vaccine only if the tests produce no evidence of the presence of B virus, Mycobacterium tuberculosis and SV₄₀.

(2) Each single strain virus pool shall be shown to be free of lymphocytic choriomeningitis virus and other mouse pathogens by intracerebral injection into 10 or more mice which shall be observed daily for at least 21 days. All mice which die during the observation period shall be studied as to the possible cause of death. A negative test shall not be valid unless at least 8 mice survive the full observation period and unless the virus pool was found free of agents pathogenic for mice; and

(3) An identity test shall be done on each virus pool using monovalent adenovirus serums free from poliomyelitis antibodies. Such serums shall

¹ Sec. 73.132(a) (1) amended and effective 3/5/63, 28 FR 2110.

have been prepared from animals immunized with virus grown in other than the tissue used for the neutralization test. The identity tests shall be done (i) in monkey kidney and (ii) in HeLa or other equally susceptible cells. The tissue cultures shall be observed for 7 days. Those showing cytopathogenic effect in the presence of type specific serum shall be subcultured in monkey kidney cells or HeLa cells. The subcultures shall be maintained for 7 days and observed for cytopathogenic effect. Only virus pools free of unidentified cytopathogenic agents and free of all viruses pathogenic to man other than adenoviruses may be used for vaccine manufacture.

(b) *Single strain tissue culture test for adenovirus.* (1) The samples specified in § 73.131(d) shall be placed immediately after sampling in contact with sodium bisulfite or a similar formaldehyde neutralizing substance that will stop the inactivation process. Each sample shall be dialyzed or rendered non-toxic to tissue culture cells by an appropriate method which does not affect the detection of live virus. An amount of fluid representing at least 5 milliliters of the original virus pool shall be inoculated into monkey kidney or other equally susceptible tissue cultures. The tissue cultures shall be maintained for 7 to 12 days and examined at intervals. At the end of the above period, the cell sheet shall be removed from each culture vessel, broken up by an appropriate means, suspended in a portion of its culture fluid equal to at least 10 percent of the volume which was present during incubation, and inoculated into corresponding fresh tissue culture preparations. Any fluids recovered prior to refeeding during original observation period shall be held at 2° to 5° C. A volume of each fluid representing at least 10 percent of the total volume shall be subcultured to fresh tissue culture. All subcultures shall be examined for at least 7 days. This test shall be considered negative only if no cellular degeneration occurs attributable to any virus.

(2) A sample of at least 500 milliliters of each single strain pool shall be fully subjected to the following testing procedure in tissue culture cells, with half the sample in monkey kidney cells and half in suitable human cells of demonstrated high susceptibility to adenovirus and poliovirus. The entire sample shall be dialyzed and rendered non-toxic for tissue culture cells. Each half of the

sample shall be inoculated into 4 or more tissue culture bottles of suitable capacity so that direct observation of the culture cells is possible under conditions which assure the growth of adenovirus, poliovirus or simian viruses should infective particles of any one of these viruses be present in the vaccine. The monkey kidney cell cultures shall be performed as described in § 73.102(b) except that a third subculture shall be included after 21 days of incubation of the initial culture and that this subculture shall be made by suspending the cell sheet. The initial human cell cultures shall be observed for at least 12 days. A subculture shall be made on each fluid at each refeeding and on the suspension of each cell sheet in the culture fluid removed at the end of the observation period. The inoculum for the subcultures shall be a volume of at least 2 percent of that of the fluid being studied. The subculture shall be examined frequently and refed as required, and maintained for a period of at least 12 days. If a cytopathogenic effect occurs during the test, the vaccine pool shall be held until the matter is resolved. If active poliomyelitis virus or adenovirus is indicated, the strain pool shall not be used for inclusion in a final vaccine. If other viruses are present, the pool shall not be used unless it can be demonstrated that such viruses did not originate from the strain pool being tested.

(c) *Final vaccine pool tissue culture test.* No less than 1,500 milliliters of the final vaccine pool without final preservative, prepared by pooling the individual single strain preparations, shall be tested in accordance with § 73.102 (b) and (c).

(d) *Final vaccine test for active virus in monkeys.* Final bulk vaccine shall be tested in monkeys as prescribed in § 73.102(e) except that the test may be applied to vaccine before it is placed in final containers, and the sample may be dialyzed in order to remove sodium bisulfite or the sodium bisulfite formaldehyde complex before injection intraspinally and intracerebrally into monkeys. In no case, however, shall dialyzed vaccine be used for the intramuscular injection of the monkeys. The test is considered negative if the histological and other studies leave no doubt that virus infections attributable to the vaccine did not occur.

(e) *Exclusion of certain pools from final vaccine.* Pools which fail to pass the tissue culture safety tests prescribed in this section shall not be included in final vaccine, unless it can be clearly

shown that the cytopathogenic agent occurred in the test system and not in the vaccine pool. No pool shall be subjected to reprocessing.

§ 73.133 Potency test.

Each lot of vaccine shall be subjected to a potency test which permits an estimation of the antigenic capacity of the vaccine in comparison with a reference vaccine distributed by the National Institutes of Health. This shall be done using at least 6 animals for each dilution of each vaccine tested and measuring the neutralizing antibody response of the animals receiving test vaccine and others receiving reference vaccine in simultaneous tests. The average antibody level for each type shall equal or exceed the corresponding value of the reference vaccine.

§ 73.134 General requirements.

(a) *Separate facilities.* The personnel, equipment and supplies used in the manufacture of adenovirus vaccine shall be separated from personnel, equipment or supplies used in connection with any other pathogenic virus to the extent necessary to prevent cross-contamination.

(b)¹ *Final container tests.* Tests shall be made on final containers for safety, sterility and identity, in accordance with §§ 73.72, 73.73 and 73.75 respectively.

(c) *Release of vaccine.* A lot of vaccine shall not be released unless all required safety tests have given negative results.

(d) *Extraneous protein.* Extraneous protein capable of producing allergenic effects on human subjects shall not be added to the final virus production medium. If animal serum is used at any stage, its calculated concentration in the final medium shall not exceed 1:1,000,000.

(e) *Nitrogen content.* The final vaccine shall have a protein nitrogen content of less than 0.02 milligram per milliliter.

(f) *Dose.* These additional standards for adenovirus vaccine are based on a human dose not exceeding 1.0 milliliter for a single injection.

(g) *Labeling.* In addition to compliance with the requirements of §§ 73.50 to 73.55, inclusive, the

label or package enclosure shall include an appropriate statement indicating the type and amount of each antibiotic added, if any. The preservative used shall be stated on the label, as well as allergenic substances added, if any, and the source, composition, and method of inactivation of the viruses.

(h)² *Dating.* The expiration date shall be not more than 6 months after either the date of manufacture, as defined in § 73.83(a), or the date of issue from cold storage. Such date of issue shall be not more than 6 months after such date of manufacture, and the product prior to issue shall be kept constantly at a temperature not exceeding 10° C.

(i) *Requirements for samples and protocols.* For each lot of vaccine, the following material shall be submitted to the Director, Division of Biologics Standards, National Institutes of Health, Bethesda, Maryland 20014:

(1) A 2,500-milliliter sample of the final vaccine taken at the latest possible stage of manufacture before the addition of preservative.

(2) A 200-milliliter bulk sample of the final vaccine containing all preservatives.

(3) A total of at least 200-milliliter sample of the final vaccine in final labeled containers.

(4) Protocols showing the history of the lot and the results of all of the tests which were carried out by the manufacturer.

§ 73.135 Equivalent methods.

The provisions of § 73.105, permitting modifications in methods if found equivalent in assuring safety, purity, and potency, shall be applicable to the additional standards relating to adenovirus vaccine (§§ 73.130 to 73.134, inclusive).

ADDITIONAL STANDARDS: MEASLES VIRUS VACCINE, LIVE, ATTENUATED

§ 73.140 The product.

(a) *Proper name and definition.* The proper name of this product shall be Measles Virus Vaccine, Live, Attenuated, which shall consist of a preparation of live, attenuated, measles virus.

¹ Sec. 73.134(b) amended June 28, 1961, 26 FR 5755, to become effective July 28, 1961.

² Sec. 73.134(h) amended June 28, 1961, 26 FR 5755, to become effective July 28, 1961.

(b)¹ *Criteria for acceptable strains of attenuated measles virus.* Strains of attenuated measles virus used in the manufacture of vaccine shall be identified by (1) historical records including origin and manipulation during attenuation, (2) antigenic specificity as measles virus as demonstrated by tissue culture neutralization tests. Strains used for the manufacture of Measles Virus Vaccine, Live, Attenuated, shall have been shown to be safe and potent in man by field studies with experimental vaccines. Vaccine prepared from measles virus strains propagated in chick embryo or canine renal tissue cultures shall have been demonstrated as safe and potent in at least 10,000 susceptible persons. Susceptibility shall be shown by the absence of neutralizing or other antibodies against measles virus, or by other appropriate methods. Vaccine prepared from measles virus strains propagated in canine renal tissue cultures shall also have been demonstrated to be free from harmful effects in not less than 100,000 persons. Seed virus used for vaccine manufacture shall be free of all demonstrable extraneous viable microbial agents.

(c) *Neurovirulence safety test of the virus seed strain in monkeys*—(1) *The test.* A demonstration shall be made in monkeys of the lack of neurotropic properties of the seed strain of attenuated measles virus used in manufacture of measles virus vaccine. For this purpose, vaccine from each of the five consecutive lots (§ 73.145) used by the manufacturer to establish consistency of manufacture of the vaccine, shall be tested for neurovirulence in the following manner:

Samples of each of the five lots of vaccine shall be tested in measles susceptible monkeys. Immediately prior to initiation of a test each monkey shall have been shown to be serologically negative for neutralizing antibodies by means of a tissue culture neutralization test with undiluted serum from each monkey tested at approximately 100 TCID₅₀ of Edmonston strain measles virus, or negative for measles virus antibodies as demonstrated by tests of equal sensitivity. Each lot of vaccine shall be tested in ten monkeys by the intracerebral inoculation of 0.5 ml. into the thalamic region of each hemisphere and an inoculation of 0.25 ml. intracisternally. The combined dose of measles virus inoculated into the central nervous system of each monkey shall be no less than the equivalent of 1,000 TCID₅₀ of the NIH standard (§ 73.141(d)). The monkeys shall be observed for 17 to 21 days and symptoms of paralysis as well as other evidences of neurological disorders shall be recorded. The test must be re-

peated if more than 20 percent of a group of monkeys die from nonspecific causes. Animals which die within the first 48 hours of initiation of the test may be replaced. At the end of the observation period each surviving monkey shall (a) be bled and the serum tested for evidence of serum antibody conversion to measles virus and (b) be autopsied and histopathological sections prepared of appropriate areas of the brain, and the sections examined microscopically for evidence of central nervous system involvement.

(2) *Wild virus controls.* As a check against the inadvertent introduction of wild measles virus, at least four uninoculated measles susceptible control monkeys shall be maintained as either cage mates to, or within the same immediate area of, the ten inoculated test animals for each lot of vaccine for the entire period of observation (17–21 days) and an additional ten days. Serum samples from these control contact monkeys drawn at the time of seed virus inoculation of the test animals, and again after completion of the test, shall be shown to be free of measles neutralizing antibodies.

(3) *Test results.* (i) For each lot of vaccine under test, at least 80 percent of the monkeys must show measles antibody serological conversion (1:4 or greater) and the control contact monkeys must demonstrate no immunological response indicative of measles virus infection.

(ii) The measles virus seed has acceptable neurovirulence properties for use in vaccine manufacture if for each of the five lots (a) 80 percent of the monkeys survive the observation period and (b) there is no clinical or histopathological evidence of central nervous system involvement attributable to the inoculated virus.

(4) *New seed lots—test for neurovirulence.* The neurovirulence properties of each new seed shall be tested as prescribed in this paragraph (c). Only seed lots which meet the neurovirulence requirement shall be used for Measles Virus Vaccine manufacture. The test need not be repeated as long as the same seed lot of virus is used.

§ 73.141 Manufacture of Live, Attenuated Measles Virus Vaccine.²

(a) *Virus cultures.* Virus shall be propagated in chick embryo tissue cultures or canine renal tissue cultures.

² Sec. 73.141(a) amended, new (b) and (c) added, and amended, and old (b) through (f), redesignated as (d) through (h), 10/22/63, 23 FR 11269.

¹ Sec. 73.140(b) amended 10/22/63, 28 FR 11269.

(b) *Virus propagated in chick embryo tissue cultures.* Embryonated chicken eggs used as the source of chick embryo tissue for the propagation of measles virus shall be derived from flocks certified to be free of *Salmonella pullorum*, avian tuberculosis, fowl pox, Rous sarcoma, avian leucosis and other adventitious agents pathogenic for chickens. If eggs are procured from flocks that are not so certified, tests shall be performed to demonstrate freedom of the vaccine from such agents. (See § 73.142(a)(8) for test for avian leucosis.)

(c) *Virus propagated in canine renal tissue cultures.* Only dogs in overt good health which have been maintained in quarantine in vermin-proof quarters for a minimum of six months, having had no exposure to other dogs or animals throughout the quarantine period, or dogs born to dogs while so quarantined, provided the progeny have been kept in the same type of quarantine continuously from birth, shall be used as a source of kidney tissue for the propagation of measles virus.

(1) *Dogs used for experimental purposes.* Dogs that have been used previously for experimental or testing purposes with microbiological agents shall not be used as a source of kidney tissue in the manufacture of vaccine.

(2) *Quarantine and necropsy.* Each dog shall be examined periodically during the quarantine period as well as at the time of necropsy under the direction of a qualified pathologist, physician or veterinarian having experience with diseases of dogs, for the presence of signs or symptoms of ill health, particularly for evidence of tuberculosis, infectious canine hepatitis, canine distemper, rabies, leptospirosis, and other diseases indigenous to dogs. If there are any such signs, symptoms, or other significant pathological lesions observed, tissue from such animals shall not be used in the manufacture of Measles Virus Vaccine, Live, Attenuated.

(d) *NIH reference measles virus.* An NIH Reference Measles Virus, Live, Attenuated, shall be obtained from the Division of Biologics Standards as a control for correlation of virus titers.

(e) *Passage of virus strain in vaccine manufacture.* Virus in the final vaccine shall represent no more than ten tissue culture passages beyond the passage used to perform the clinical trials

(§ 73.140(b)) which qualified the manufacturer's vaccine strain for license.

(f) *Tissue culture preparation.* Only primary cell tissue cultures shall be used in the manufacture of Measles Virus Vaccine. Continuous cell lines shall not be introduced or propagated in Measles Virus Vaccine manufacturing areas.

(g) *Control vessels.* (1) From the tissue used for the preparation of tissue cultures for growing attenuated measles virus, an amount of processed cell suspension equivalent to that used to prepare 500 ml. of tissue culture shall be used to prepare uninfected tissue control materials. This material shall be distributed in control vessels and observed microscopically for a period of no less than 14 days beyond the time of inoculation of the production vessels with measles virus; but if the production vessels are held for use in vaccine manufacture for more than 14 days, the control vessels shall be held and observed for the additional period. At the end of the observation period or at the time of virus harvest, whichever is later, fluids from the control cultures shall be tested for the presence of adventitious agents as follows:

Samples of fluid from each control vessel shall be collected at the same time as fluid is harvested from the corresponding production vessels. If multiple virus harvests are made from the same cell suspension, the control samples for each harvest shall be frozen and stored at -60°C . until the last viral harvest for that cell suspension is completed. The fluid from all the control samples from that suspension shall be pooled in proportionate amounts and at least five ml. inoculated into human and simian cell tissue culture systems and in the tissue culture system used for virus production. The cultures shall be observed for the presence of changes attributable to growth of adventitious viral agents including hemadsorption viral agents.

(2) The cell sheets of one quarter to one third of the control vessels shall be examined at the end of the observation period (14 days or longer) for the presence of hemadsorption viruses by the addition of guinea pig red blood cells. If the chick embryo cultures were not derived from a certified source (§ 73.141(b)), the remaining tissue culture controls may be used to test for avian leucosis virus using either Rubin's procedure for detecting Re-

sistance Inducing Factor (RIF) or a method of equivalent effectiveness.

(3) The test is satisfactory only if there is no evidence of adventitious viral agents and if at least 80 percent of the control vessels are available for observation at the end of the observation period (14 days or longer).

(h) *Test samples.* Samples of virus harvests or pools for testing by inoculation into animals, into tissue culture systems, into embryonated hens' eggs, and into bacteriological media, shall be withdrawn immediately after harvesting or pooling but prior to freezing except that samples of test materials frozen immediately after harvesting or pooling and maintained at -60°C . or below, may be tested upon thawing, provided no more than two freeze-thaw cycles are employed. The required tests shall be initiated without delay after thawing.

§ 73.142 Test for safety.

(a)¹ *Tests prior to clarification of vaccine manufactured in chick embryo tissue cultures.* Prior to clarification, the following tests shall be performed on each virus pool of chick embryo tissue culture:

(1) *Inoculation of adult mice.* Each of at least 20 adult mice each weighing 15–20 grams shall be inoculated intraperitoneally with 0.5 ml. and intracerebrally with 0.03 ml. amounts of each virus pool to be tested. The mice shall be observed for 21 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection by direct observation and subinoculation of appropriate tissue into at least five additional mice which shall be observed for 21 days. The virus pool may be used only if at least 80 percent of the original group of mice remain healthy and survive the observation period and if none of the mice show evidence of a transmissible agent or other viral infection, other than measles virus, attributable to the vaccine.

(2) *Inoculation of suckling mice.* Each of at least 20 suckling mice less than 24 hours old shall be inoculated intracerebrally with 0.01 ml. and intraperitoneally with 0.1 ml. of the virus pool to

be tested. The mice shall be observed daily for at least 14 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection. Such examination shall include subinoculation of appropriate tissue suspensions into an additional group of at least five suckling mice by intracerebral and intraperitoneal routes and observed daily for 14 days. In addition, a blind passage shall be made of a single pool of the emulsified tissue (minus skin and viscera) of all mice surviving the original 14-day test. The virus pool is satisfactory for Measles Virus Vaccine only if at least 80 percent of the original inoculated mice remain healthy and survive the entire observation period, and if none of the mice used in the test show evidence of a transmissible agent or viral infection, other than measles virus, attributable to the vaccine.

(3) *Inoculation of monkey tissue cell cultures.* A volume of virus suspension of each undiluted virus pool, equivalent to at least 500 human doses or 50 ml., whichever represents a greater volume, shall be tested for adventitious agents in cercopithecus monkey kidney tissue culture preparations, after neutralization of the measles virus by a high titer antiserum of nonhuman, nonsimian, and nonchicken origin. The immunizing antigen used for the preparation of the measles antiserum shall be grown in tissue culture cells that shall be free of extraneous viruses which might elicit antibodies that could inhibit growth of extraneous viruses present in the measles virus pool. The tissue culture of the virus pool shall be observed for no less than 14 days. The virus pool is satisfactory for measles virus vaccine only if all the tissue culture tests fail to show evidence of any extraneous transmissible agent other than measles virus attributable to the vaccine.

(4) *Inoculation of other cell cultures.* The measles virus pool shall be tested in the same manner as prescribed in subparagraph (3) in rhesus or cynomolgus monkey kidney, chick embryo, and human tissue cell cultures.

(5) *Inoculation of embryonated chicken eggs.* A volume of virus suspension of each undiluted virus pool, equivalent to at least 100 doses or 10 ml., whichever represents a greater volume, after neutralization of the measles virus by a high titer antiserum of nonhuman, nonsimian, nonchicken

¹ Sec. 73.142(a) amended, new (b) added and old (b) and (c) redesignated as (c) and (d), 10/22/63, 28 FR 11269.

origin, shall be tested in embryonated eggs by the allantoic cavity route of inoculation and a separate group tested by the yolk sac route of inoculation, using 0.5 ml. of inoculum per egg. The virus pool is satisfactory if there is no evidence of adventitious agents.

(6) *Test for Pleuropneumonia-like Organisms (PPLO (Mycoplasma))*. Each chick virus pool shall be tested for the presence of PPLO (Mycoplasma). Samples of the virus for this test shall be stored either (i) at 2–5° C. for not longer than 24 hours, or (ii) at –20° C. or lower if stored for longer than 24 hours. The PPLO test shall be performed both on samples of viral harvests and control fluids as follows: No less than 2.0 ml. of a sample shall be inoculated in no less than 0.1 ml. amounts evenly distributed over the surface of 20 agar plates. No less than 1.0 ml. of sample shall be inoculated into each of four tubes of 10 ml. each of broth. Ten agar plates and two tube cultures shall be incubated aerobically at 36° C. $\pm 1^\circ$ C. and the remaining agar plates and tube cultures shall be incubated anaerobically at 36° C. $\pm 1^\circ$ C. in an environment of 5% CO₂ and 95% N₂. The aerobic broth cultures shall be incubated for no less than three days and no more than five days, at which time 0.5 ml. from each of two tubes shall be combined and the 1.0 ml. subinoculated to ten additional agar plates. The anaerobic broth cultures shall be tested in the same manner. All inoculated agar plate cultures shall be incubated for no less than 14 days when observation for PPLO growth shall be made at a magnification of no less than 300X. If the Dienes Methylene Blue-Azure dye or equivalent staining procedure is used, no less than a one square cm. plug of the agar shall be excised from the inoculated area and examined for the presence of PPLO colonies. Control cultures of known strains of PPLO shall be included in the test. Identification of the PPLO shall be made by comparison of the growth obtained from the test sample with the controls, with respect to typical colonial and microscopic morphology. The virus pool is satisfactory for measles virus vaccine only if none of the tests on the samples of viral harvests show evidence of the presence of the Pleuropneumonia-like organism (Mycoplasma).

(7) *Bacteriological tests*. Each virus pool shall be tested for sterility in accordance with

§ 73.73. In addition each virus pool shall be tested for the presence of *M. tuberculosis*, both avian and human, by appropriate culture methods.

(8) *Test for avian leucosis*. If the cultures were not derived from a certified source (§ 73.141(b)), and the control fluids were not tested for avian leucosis (§ 73.141(g)), at least 500 doses or 50 ml., whichever represents a greater volume of each undiluted vaccine pool, shall be tested and found negative for avian leucosis, using either Rubin's procedure for detecting Resistance Inducing Factor (RIF) or another method of equivalent effectiveness.

(b) *Tests prior to clarification of vaccine manufactured in canine renal tissue cultures*. Prior to clarification, the following tests shall be performed on each virus pool of canine renal tissue culture:

(1) *Inoculation of adult mice*. Virus grown in canine renal tissue cultures shall be tested in adult mice, as prescribed in paragraph (a)(1) of this section for virus grown in chick embryo tissue cultures. Test result standards are those prescribed therein.

(2) *Inoculation of suckling mice*. Each of at least 20 suckling mice less than 24 hours old shall be inoculated intracerebrally with 0.01 ml. and intraperitoneally with 0.1 ml. of the canine renal tissue culture virus pool to be tested. The mice shall be observed daily for at least 28 days. Each mouse that dies after the first 48 hours of the test, or is sacrificed because of illness, shall be necropsied and all areas examined for evidence of viral infection. Such examination shall include subinoculation of appropriate tissue suspensions into an additional group of at least five suckling mice by intracerebral and intraperitoneal routes and observed daily for 28 days. The virus pool is satisfactory for Measles Virus Vaccine only if at least 80 percent of the originally inoculated mice remain healthy and survive the entire observation period, and if none of the mice used in the test show evidence of having been infected with rabies virus or any other transmissible agent or viral infection other than measles virus.

(3) *Inoculation of monkey tissue cell cultures*. Virus grown in canine renal tissue cultures shall be tested in monkey tissue cell cultures as prescribed in paragraph (a)(3) of this section for virus grown in chick embryo tissue cultures. Test

result standards are those prescribed therein.

(4) *Inoculation of other cell cultures.* Virus grown in canine renal tissue cultures shall be tested in rhesus or cynomolgus monkey kidney tissue, canine renal tissue and human tissue cell cultures as prescribed in paragraph (a)(3) of this section for testing virus grown in chick embryo tissue culture in cercopithecus monkey kidney tissue culture preparations. Test result standards are those prescribed therein.

(5) *Inoculation of embryonated eggs.* Virus grown in canine renal tissue cultures shall be tested in embryonated eggs as prescribed in paragraph (a)(5) of this section for virus grown in chick embryo tissue cultures. Test result standards are those prescribed therein.

(6) *Test for Pleuropneumonia-like organisms. (PPLO). (Mycoplasma).* Virus grown in canine renal tissue cultures shall be tested for Pleuropneumonia-like organisms as prescribed in paragraph (a)(6) of this section for virus grown in chick embryo tissue cultures. Test result standards are those prescribed therein.

(7) *Bacteriological test.* Each virus pool shall be tested for sterility in accordance with § 73.73. In addition each virus pool shall be tested for *M. tuberculosis*, human, by appropriate culture methods.

(8) *Tests for adventitious agents.* Each virus pool shall be tested for the presence of such adventitious agents as canine distemper virus, canine hepatitis virus, leptospira and toxoplasma and the following fungi: coccidiomyces, histoplasma and blastomyces. The virus pool is satisfactory only if the results of all tests show no evidence of any extraneous agent attributable to the canine renal tissue or the vaccine.

(c) *Clarification.* After harvesting and removal of samples for testing as prescribed above in this section, the virus fluids shall be clarified by centrifugation, by passage through filters of sufficiently small porosity, or by any other method that will assure removal of all intact tissue cells which may have been collected in the harvesting process.

(d) *Test after clarification—Neurovirulence safety test in monkeys for neurotropic agents.* Before final dilution for standardization for live measles virus content each lot of measles virus vaccine shall be tested for neurotropic agents fol-

lowing the procedure prescribed in § 73.102(e), except that antibody determinations for measles need not be performed, the test shall be performed before the product is placed in final containers and prior to the addition of an adjuvant, and that symptoms suggestive of any neurotropic agent, rather than those specifically suggestive of poliomyelitis, shall be recorded during the observation of 17 to 19 days. The lot is satisfactory only if the histological and other studies produce no clinical or histological evidence of central nervous system involvement attributable to the presence of a neurotropic agent in the vaccine.

§ 73.143 Potency test.

The concentration of live measles virus shall constitute the measure of potency. The titration shall be performed in a suitable cell culture system, free of wild viruses, using either the NIH reference measles virus, live, attenuated or a calibrated equivalent strain as a titration control. The concentration of live measles virus contained in the vaccine of each lot under test shall be no less than the equivalent of 1,000 TCID₅₀ of the NIH reference per human dose.

§ 73.144 General requirements.

(a) *Final container tests.* Tests shall be made on final containers for safety, sterility and identity as prescribed in §§ 73.72, 73.73, and 73.75, respectively, except that an immunological and virological identity test need not be performed on the final container if it was performed on each pool or the bulk vaccine prior to filling.

(b) *Extraneous protein.* Extraneous protein, capable of producing allergenic effects on injection into human subjects, shall not be added to the vaccine at any time. If animal serum is used at any manufacturing stage, its calculated concentration in the final medium shall not exceed 1:1,000,000.

(c) *Antibiotics.* Virus for manufacturing vaccine may be grown in a medium containing minimum concentrations of suitable antibiotics except that penicillin shall not be used in the tissue culture medium or added to the final product.

(d) *Dose.* These standards are based on an individual human immunizing dose of no less than

1,000 TCID₅₀ of Measles Virus Vaccine, Live, Attenuated, expressed in terms of the assigned titer of the NIH reference measles virus.

(e) *Labeling.* Labeling shall be in compliance with §§ 73.50 to 73.55 inclusive, and the label or a package enclosure shall state the identification and source of the virus contained in the vaccine and the tissue medium in which the virus was propagated.

(f) *Samples and protocols.* For each lot of vaccine, the following materials shall be submitted to the Director, Division of Biologics Standards, National Institutes of Health, Bethesda, Maryland 20014:

(1) Protocols showing the history of manufacture of each lot of vaccine, and all results of all tests prescribed in these additional standards.

(2) A total of no less than a 500 ml. sample of bulk vaccine or an equivalent sample prior to addition of any preservative, stabilizer or adjuvant, in the frozen state (-60°C.) prior to filling into final containers.

(3) A total of no less than 200 recommended human doses of the vaccine in final labeled containers.

§ 73.145 Clinical trials to qualify for license.

To qualify for license, the antigenicity of the vaccine shall have been determined by clinical trials of adequate statistical design, by subcutaneous administration of the product. Such clinical trials shall be conducted with five consecutive lots of measles virus vaccine which have been manufactured by the same methods, each of which has shown satisfactory results in all prescribed tests. There shall be a demonstration under circumstances wherein adequate clinical and epidemiological surveillance of illness has been maintained to show that the Measles Virus Vaccine, when administered as recommended by the manufacturer—i.e., either with or without human gamma globulin—is free of harmful effect upon administration to approximately 1,000 susceptible individuals, in that there were no detectable neutralizing antibodies before vaccination and there was serological conversion after vaccination. The five lots of vaccine used to qualify for consistency of vaccine manufacture shall be distributed as evenly as possible among the 1,000 individuals tested.

Demonstration shall be made of immunogenic effect by the production of specific measles neutralizing antibodies (i.e., sero-conversion less than 1:4 to 1:8 or greater) in at least 90 percent of each of five groups of measles susceptible individuals, each having received the parenteral administration of a virus vaccine dose which is not greater than that which was demonstrated to be safe in field studies (§ 73.140(b)) when used under comparable conditions.

§ 73.146 Equivalent methods.

Modification of any particular manufacturing method or process or the conditions under which it is conducted as set forth in the additional standards relating to Measles Virus Vaccine, Live, Attenuated, shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide assurances of the safety, purity, and potency of the vaccine that are equal to or greater than the assurances provided by such standards, and the Surgeon General so finds and makes such finding a matter of official record.

ADDITIONAL STANDARDS: MEASLES VIRUS VACCINE, INACTIVATED

§ 73.150 The product.

(a) *Proper name and definition.* The proper name of this product shall be Measles Virus Vaccine, Inactivated. The vaccine shall consist of a preparation of measles virus inactivated by an appropriate method.

(b)¹ *Criteria for acceptable strains of measles virus.* Strains of measles virus used in the manufacture of vaccine shall be identified by (1) historical records including origin and manipulation and (2) antigenic specificity as measles virus as demonstrated by tissue culture neutralization tests. Strains used for the manufacture of Measles Virus Vaccine, Inactivated, shall have been shown to be safe and potent in man by field studies with experimental vaccines. Vaccine prepared from measles virus strains propagated in chick embryo tissue cultures, monkey kidney tissue cultures or canine renal tissue cultures, shall have been dem-

¹ Sec. 73.150(b) amended 10/22/63, 28 FR 11270.

onstrated as safe and potent in at least 10,000 susceptible persons. Susceptibility shall be shown by the absence of neutralizing or other antibodies against measles virus, or by other appropriate methods. Vaccine prepared from measles virus strains propagated in canine renal tissue cultures shall also have been demonstrated to be free from harmful effects in not less than 100,000 persons. Seed virus used for vaccine manufacture shall be free of all demonstrable extraneous viable microbial agents.

§ 73.151 Manufacture of Measles Virus Vaccine, Inactivated.

(a)¹ *Virus cultures.* Virus shall be propagated in chick embryo tissue cultures, monkey kidney tissue cultures, or canine renal tissue cultures.

(b) *Virus propagated in chick embryo tissue cultures.* Embryonated chicken eggs used as a source of chick embryo tissue for the propagation of measles virus shall be derived from flocks certified to be free of *Salmonella pullorum* and avian tuberculosis, fowl pox, Rous sarcoma, avian leucosis and other adventitious agents pathogenic for chickens. If eggs are procured from flocks that are not so certified, tests shall be performed to demonstrate that the virus pool be free from such agents prior to inactivation.

(c) *Virus vaccine propagated in monkey kidney tissue cultures.* Only monkeys in overt good health, that have reacted negatively to tuberculin at the start of the prescribed quarantine period shall be used as the source of kidney tissue for the manufacture of Measles Virus Vaccine, Inactivated.

(1) *Monkeys used for experimental purposes.* Monkeys that have been used previously for experimental purposes with microbiological agents shall not be used as a source of kidney tissue for the manufacture of vaccine. Monkeys that have been used previously for other experimental purposes may be used upon their return to a normal condition.

(2) *Quarantine.* Only monkeys that during the quarantine period, as provided by § 73.36(f) (2), have been tested with and have reacted negatively

to tuberculin shall be used as a source of kidney tissue for vaccine manufacture.

(3) *Necropsy.* Each animal at necropsy shall be examined under the direction of a qualified pathologist, physician or veterinarian having experience with diseases of monkeys, for the presence of signs or symptoms of ill health, particularly for (1) evidence of tuberculosis, (2) presence of herpes-like lesions, including eruptions or plaques on or around the lips, in the buccal cavity or on the gums and (3) signs of conjunctivitis. If any such signs or other significant gross pathological lesions are present, the kidney shall not be used in the manufacture of Measles Virus Vaccine, Inactivated.

(d) *Virus propagated in canine renal tissue cultures.* Only dogs in overt good health which have been maintained in quarantine in vermin-proof quarters for a minimum of six months, having had no exposure to other dogs or animals throughout the quarantine period, or dogs born to dogs while so quarantined, provided the progeny have been kept in the same type of quarantine continuously from birth, shall be used as a source of kidney tissue for the propagation of measles virus.

(1) *Dogs used for experimental purposes.* Dogs that have been used previously for experimental or testing purposes with microbiological agents shall not be used as a source of kidney tissue in the manufacture of vaccine.

(2) *Quarantine and necropsy.* Each dog shall be examined periodically during the quarantine period as well as at the time of necropsy under the direction of a qualified pathologist, physician or veterinarian having experience with diseases of dogs, for the presence of signs or symptoms of ill health, particularly for evidence of tuberculosis, infectious canine hepatitis, canine distemper, rabies, leptospirosis, and other diseases indigenous to dogs. If there are any such signs, symptoms, or other significant pathological lesions observed, the kidneys from such animals shall not be used in the manufacture of Measles Virus Vaccine, Inactivated.

(e) *NIH Reference Measles Virus.* The following NIH reference viruses shall be obtained from the Division of Biologics Standards:

(1) NIH Reference Measles Virus for titration.

(2) NIH Reference Measles Vaccine for potency testing.

¹ Sec. 73.151(a) amended, (d) through (f) redesignated as (1) through (3); new (d) added, and (g) through (k) redesignated as (e) through (i) 10/22/63, 28 FR 11270.

(f) *Passage of virus strain in vaccine manufacture.* Virus in the final vaccine shall represent no more than ten tissue culture passages beyond the passage used to perform the clinical trials which qualified the vaccine strain for license (§ 73.150(b)), and the virus of that passage shall represent vaccine that shall have met the following criteria of acceptability:

(1) *Clinical safety.* The vaccine shall be free from harmful effects. Freedom from harmful effects shall be demonstrated by administration, as recommended by the manufacturer, and while maintaining adequate clinical and epidemiological surveillance of illness, to approximately 1,000 individuals, having no detectable neutralizing antibodies before vaccination and showing serological conversion after vaccination. Five consecutive lots of vaccine shall be used to qualify the vaccine for license and shall be distributed as evenly as possible among the 1,000 individuals tested.

(2) *Clinical potency.* The immunogenic effect (i.e., sero-conversion less than 1:4 to 1:8 or greater) shall be demonstrated in at least 90 percent of each of five groups of measles susceptible individuals, each group receiving vaccine from one of the five consecutive lots of vaccine which were used to qualify the vaccine for license, and each of which shall have met the safety standards prescribed in these regulations. The dose of vaccine shall be no greater than that which was demonstrated to be safe pursuant to subparagraph (1) and the vaccine shall be used under comparable conditions.

(g) *Types of tissue culture preparation permissible.* Measles Virus Vaccine, Inactivated, shall be produced only in primary cell tissue culture. Continuous line cells shall not be used and shall not be introduced into vaccine production areas.

(h) *Use of antibiotics.* Virus for manufacturing vaccine may be grown in cultures which contain minimum concentration of suitable antibiotics except that penicillin shall not be used in the tissue culture medium or added to the final product.

(i) *Clarification.* After harvesting, the virus fluids shall be clarified by centrifugation, by passage through filters of sufficiently small porosity, or by any other method that will assure removal of all intact tissue cells which may have been collected in the harvesting process.

§ 73.152 Test for safety.

(a) *Test prior to the inactivation process.* Samples of virus pools for testing by inoculation into animals or into bacteriological media shall be withdrawn immediately after pooling but prior to freezing or further processing, and tested, prior to the inactivation process, as provided in paragraphs (b) and (c) of this section except that samples of test materials frozen immediately after pooling and maintained at -60°C . or below, may be tested upon thawing, provided no more than two freeze thaw cycles are employed. The required tests shall be conducted without delay after thawing.

(1) *Measles virus propagated in chick embryo tissue cultures—(i) Inoculation of adult mice; test for adventitious agents.* Each chick embryo virus pool shall be shown to be free of contaminating agents pathogenic for mice by the intracerebral inoculation of 0.03 ml. and intraperitoneal inoculation of 0.5 ml. amounts of the pool into each of ten or more adult mice (15–20 gms.). The mice shall be observed for at least 21 days. The virus pool is satisfactory for measles virus vaccine only if at least 80 percent of the inoculated animals survive the observation period and none of the animals inoculated shows evidence of infection with extraneous transmissible agents attributable to the vaccine.

(ii) *Test for Pleuropneumonia-like Organisms (PPLO (Mycoplasma)).* Each chick virus pool shall be tested for the presence of PPLO (Mycoplasma). Samples of the virus for this test shall be stored either (1) at $2-5^{\circ}\text{C}$. for not longer than 24 hours, or (2) at -20°C . or lower if stored for longer than 24 hours. The PPLO test shall be performed both on samples of viral harvests and control fluids as follows: No less than 2.0 ml. of the sample shall be inoculated in no less than 0.1 ml. amounts evenly distributed over the surface of 20 agar plates. No less than 1.0 ml. of the sample shall be inoculated into each of four tubes of 10 ml. each of broth. Ten agar plates and two tube cultures shall be incubated aerobically at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$. and the remaining agar plates and tube cultures shall be incubated anaerobically at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$. in an environment of 5% CO_2 and 95% N_2 . The aerobic broth cultures shall be incubated for no less than three days and no more than

five days at which time 0.5 ml. from each of the two tubes shall be combined and this 1.0 ml. subinoculated in 0.1 ml. amounts evenly distributed over the surface of 10 additional agar plates. The anaerobic broth cultures shall be incubated and subinoculated in the same manner. All inoculated agar plate cultures shall be incubated for no less than 14 days when observation for PPLO growth shall be made at a magnification of no less than 300X. If the Dienes Methylene Blue-Azure dye or equivalent staining procedure is used, no less than a one square cm. plug of the agar shall be excised from the inoculated area and examined for the presence of PPLO colonies. Control cultures of known strains of PPLO shall be included in the test. Identification of the PPLO shall be made by comparison of the growth obtained from the test sample with the controls, with respect to typical colonial and microscopic morphology. The virus pool is satisfactory for measles virus vaccine only if none of the tests on the samples of viral harvests produces evidence of the presence of the Pleuropneumonia-like organism (*Mycoplasma*).

(iii) *Bacteriological tests.* Each chick embryo virus pool shall be tested for bacteriological sterility in accordance with the procedures prescribed in § 73.73. In addition each virus pool shall be tested and found negative for the presence of *M. tuberculosis*, both avian and human, by appropriate culture methods.

(iv) *Test for avian leucosis.* The equivalent of at least 50 doses of final vaccine from each undiluted virus pool, or in proportionate amounts from individual harvests or subpools, shall be tested and found negative for avian leucosis, using either Rubin's procedure for detecting Resistance Inducing Factor (RIF) or a procedure of equivalent effectiveness. These tests may be performed on corresponding amounts of fluids from control vessels instead of on the undiluted virus pool or individual harvests of subpools.

(2) *Measles virus propagated in monkey kidney tissue cultures—*(i) *Inoculation of rabbits; test for B virus and other adventitious agents.* A minimum of 100 ml. of each monkey kidney virus pool shall be tested by inoculation into at least ten healthy rabbits, each weighing 1500–2500 grams. Each rabbit shall be injected intradermally at multiple sites with a total of 1.0 ml. and subcutane-

ously with 9.0 ml. of the virus, and the animals observed for at least three weeks. Each rabbit that dies after the first 24 hours of the test or is sacrificed because of illness shall be necropsied and the brain and organs removed and examined. The virus pool may be used for measles virus vaccine only if at least 80 percent of the rabbits remain healthy and survive the entire period and if none of the rabbits used in the test shows lesions of any kind at the sites of inoculation or shows evidence of B virus or any other transmissible agent attributable to the vaccine.

(ii) *Inoculation of adult mice; test for adventitious agents.* Each virus pool grown in monkey kidney tissue culture shall be tested in adult mice. The test shall be performed and the results measured against the standards prescribed in paragraph (a) (1) (i) for chick embryo tissue culture.

(iii) *Inoculation of guinea pigs; test for M. tuberculosis.* Each of at least five guinea pigs, each weighing 350–450 grams shall be inoculated intraperitoneally with 5.0 ml. of the monkey kidney virus pool to be tested. The animals shall be observed for at least 42 days for death or signs of disease. Each animal that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied. The tissues shall be examined both microscopically and culturally for evidence of *M. tuberculosis*. The virus pool is satisfactory for measles virus vaccine only if at least 80 percent of the original group of guinea pigs remain healthy and survive the observation period, and if none of the animals used in the test shows evidence of infection with *M. tuberculosis* or any extraneous transmissible agent attributable to the vaccine.

(iv) *Bacteriological tests.* Each monkey kidney virus pool shall be tested for bacteriological sterility in accordance with the procedures prescribed in § 73.73. In addition each virus pool shall be tested for the presence of *M. tuberculosis* (human) by appropriate culture methods.

(v) *Tissue culture test for SV₄₀.* Each individual harvest or virus pool, or a pool of tissue culture fluids from corresponding control vessels, shall be tested for the presence of SV₄₀ either as follows or by a test producing equally reliable results: five ml. of a measles virus pool shall be neutralized by high titer antiserum of an origin other than human, chicken or simian. The sample

shall be tested in the same tissue culture system used for propagating the virus vaccine, and in primary cercopithecus tissue cultures or in a cell line of demonstrated equal susceptibility to SV₄₀. The tissue cultures shall be observed for at least 14 days and at the end of the observation period at least one subculture of fluid shall be made in the same tissue culture system and the test continued for an additional 14 days. The virus harvest or virus pool is satisfactory for measles virus vaccine only if the test produces no evidence of the presence of SV₄₀.

(3) *Measles¹ virus propagated in canine renal tissue cultures*—(i) *Inoculation of adult mice; test for adventitious agents.* Each virus pool prepared from canine renal tissue cultures shall be shown to be free from contaminating agents pathogenic for mice by the test prescribed in subparagraph (1)(i) of this section for chick embryo virus pools. Test result standards are those prescribed therein.

(ii) *Inoculation of suckling mice.* Suckling mice shall be inoculated as prescribed in § 73.142 (b)(2) for virus (live, attenuated) grown in canine renal tissue cultures. Test result standards are those prescribed therein.

(iii) *Inoculation of monkey tissue cell cultures.* Monkey tissue cell cultures shall be inoculated as prescribed in § 73.142(a)(3) for virus (live, attenuated) grown in chick embryo tissue cultures. Test result standards are those prescribed therein.

(iv) *Inoculation of other cell cultures.* Virus grown in canine renal tissue cultures shall be tested in rhesus or cynomolgus monkey kidney tissue, canine renal tissue and human tissue cell cultures as prescribed in § 73.142(a)(3) for testing virus grown in chick embryo tissue cultures in cercopithecus monkey kidney tissue culture preparations. Test result standards are those prescribed therein.

(v) *Inoculation of embryonated chicken eggs.* Embryonated chicken eggs shall be inoculated as prescribed in § 73.142(a)(5) for virus (live, attenuated) grown in chick embryo tissue cultures. Test result standards are those prescribed therein.

(vi) *Test for Pleuropneumonia-like organisms (PPLO) (Mycoplasma).* The test for Pleuropneumonia-like organisms (PPLO) shall be per-

formed as prescribed in § 73.142(a)(6) for virus (live, attenuated) grown in chick embryo tissue cultures. Test result standards are those prescribed therein.

(vii) *Bacteriological test.* Each virus pool shall be tested for sterility in accordance with § 73.73. In addition each virus pool shall be tested for *M. tuberculosis*, human, by appropriate culture methods.

(viii) *Test for adventitious agents.* Each virus pool shall be tested for the presence of the adventitious agents enumerated in § 73.142(b)(8) for virus (live, attenuated) grown in canine renal tissue cultures. Test result standards are those prescribed therein.

(b) *Inactivation of virus.* The measles virus shall be inactivated through the use of an agent or method which the manufacturer has demonstrated to be effective in inactivating a series of at least five consecutive lots of measles virus vaccine. If formaldehyde is used for inactivation, it shall be added to the virus suspension to a final concentration of U.S.P. formaldehyde solution of at least 1:4,000. The inactivation shall be conducted under controlled conditions of pH and temperature. As an indication of inactivation, not less than two samples shall be removed at the time of inactivation, and titrated in an appropriate tissue cell culture for viable measles virus. Regardless of the concentration of formaldehyde or other inactivating agent used, the total inactivation period shall be not less than three times the period demonstrated by the manufacturer to be necessary to reduce the concentration of live virus to a point where no virus is detectable in a 5.0 ml. sample.

(c) *Tests after inactivation for viable measles virus and adventitious agents*—(1) *Test in tissue cultures.* A sample representing the equivalent of at least 500 doses of final vaccine of each lot shall be rendered nontoxic for tissue culture cells and tested as follows: One half of the sample shall be tested in the same tissue culture system used for propagating the virus vaccine and one half of the sample shall be tested in primary cercopithecus monkey kidney tissue or another suitable cell line of demonstrated high susceptibility to measles virus, poliovirus, and SV₄₀ or other adventitious viral agents. Each half of the sample shall be inoculated so that direct microscopic observation of the culture cells is possible under conditions

¹ Sec. 73.152(a), subparagraph (3) added 10/22/63, 28 FR 11269.

which assure the growth of measles virus, polio-virus, and simian viruses which might have survived the inactivation procedure. After inoculation of the test sample, the tissue cultures shall be observed for at least 14 days. At the end of the observation period the fluids from all the culture bottles in a system shall be removed and pooled. At least two percent of each pool shall be sub-inoculated in the same cell system as that from which the pooled sample was drawn. The subcultures shall be observed for a period of at least 14 days and examined for cell changes indicative of viral growth. The lot of final vaccine is satisfactory for measles virus vaccine only if none of the tissue culture tests show evidence of viable measles virus or any extraneous transmissible agents attributable to the vaccine.

(2) *Test in embryonated chicken eggs.* For vaccine produced in chick embryo tissue culture, the equivalent of at least 100 doses of each vaccine lot shall be tested in embryonated eggs by the allantoic cavity route and of 100 doses by the yolk sac route of inoculation, using 0.5 ml. of inoculum per egg, and found negative for the presence of extraneous agents in the vaccine.

(3) *Test in monkeys for neurotropic agents.* Each lot of vaccine shall be tested for neurotropic agents following the procedure prescribed in § 73.102(e) except that antibody determinations for measles need not be performed, the test shall be performed before the product is placed in final containers and prior to the addition of an adjuvant, and that symptoms suggestive of all neurotropic agents shall be recorded during the observation period of 17 to 19 days. The lot is satisfactory only if the histological and other studies produce no clinical or histological evidence of central nervous system involvement attributable to the presence of a neurotropic agent in the vaccine.

§ 73.153 Test for potency.

A potency test shall be performed on each lot of vaccine by determining the antigenic capacity of the vaccine under tests in comparison with a reference vaccine of antigenic capacity at least equal to that required for the clinical trials specified in § 73.151(h) (2). The test shall be performed using at least ten animals for each dilution of the test vaccine and of the reference vaccine. The average

antibody levels of the animals injected with the vaccine under test shall equal or exceed the average antibody levels of the animals injected with the reference vaccine.

§ 73.154 General requirements.

(a) *Final container tests.* Tests shall be made on final containers for safety, and sterility and identity in accordance with §§ 73.72, 73.73, and 73.75 respectively.

(b) *Extraneous protein.* Extraneous protein capable of producing allergenic effects on injection into human subjects shall not be added to the final virus medium. If serum is used at any stage, its calculated concentration in the final medium shall not exceed 1:1,000,000. The final vaccine shall have a protein nitrogen content of less than 0.02 milligram per individual human dose.

(c) *Dose.* These standards are based on an individual human dose of 1.0 ml. for a single injection.

(d) *Labeling.* Labeling shall be in compliance with the requirements of §§ 73.50 to 73.55 inclusive. The label or a package enclosure shall in addition state the source and method of inactivation of the virus.

(e) *Adjuvants in the final vaccine.* An adjuvant shall not be introduced into the product unless there is satisfactory evidence that it does not adversely affect the safety or potency of the vaccine. In no event shall an individual human injection contain more aluminum than that contained in 15 milligrams by assay, or 20 milligrams by calculation, of $\text{Al K}(\text{SO}_4) \cdot 12\text{H}_2\text{O}$. Aluminum in another form may be used, provided the amount of aluminum does not exceed the amount permitted as $\text{Al K}(\text{SO}_4) \cdot 12\text{H}_2\text{O}$.

(f) *Requirements for samples and protocols.*

For each lot of vaccine, the following material shall be submitted to the Director, Division of Biologics Standards, National Institutes of Health, Bethesda, Maryland 20014:

(1) A sample of 1,500 doses of the vaccine taken after the last stage of manufacture before the addition of preservative or adjuvant.

(2) A sample of 100 doses of the final vaccine containing all preservatives.

(3) A sample of 200 doses of the final vaccine in final labeled containers.

(4) Protocols showing the history of the lot and all results of all tests prescribed in these additional standards.

§ 73.155 Equivalent methods.

Modification of any particular method or process or the conditions under which it is conducted as set forth in the additional standards relating to Measles Virus Vaccine, Inactivated, shall be permitted whenever the manufacturer presents evidence that demonstrates the modification will provide assurances of the safety, purity, and potency of the vaccine that are equal to or greater than the assurances provided by such standards, and the Surgeon General so finds and makes such finding a matter of official record.

ADDITIONAL STANDARDS: WHOLE BLOOD (HUMAN)

§ 73.300 Proper name and definition.

For the purpose of section 351(a)(2) of the Act and § 73.1(k), the proper name of this product shall be Whole Blood (Human) preceded by a term or terms indicating the anticoagulant used. Whole Blood (Human) is defined as blood collected from human donors for transfusion to human recipients.

§ 73.301 Suitability of donor.

(a) *Method of determining.* The suitability of a donor as a source of Whole Blood (Human) shall be determined by a qualified physician or by persons under his supervision and trained in determining suitability. Such determination shall be made on the day of collection from the donor by means of a medical history, a test for hemoglobin level, and such physical examination as appears necessary.

(b) *Qualifications of donor; general.* Only those persons may serve as a source of Whole Blood (Human) who are in good health, as indicated in part by:

- (1) Normal temperature;
- (2) Demonstration that systolic and diastolic blood pressures are within normal limits, unless the examining physician is satisfied that an individual with blood pressures outside these limits

is an otherwise qualified donor under the provisions of this section;

(3) A blood hemoglobin level which shall be demonstrated to be no less than 12.5 gm. of hemoglobin per 100 ml. of blood;

(4) Freedom from acute respiratory diseases;

(5) Freedom from any infectious skin disease at the site of phlebotomy and from any such disease generalized to such an extent as to create a risk of contamination of the blood; and

(6) Freedom from any disease transmissible by blood transfusion, insofar as can be determined by history and examinations indicated above.

(c) *Additional qualifications of donor; viral hepatitis.* No individual shall be used as a source of Whole Blood (Human) if he has—

(1) A history of viral hepatitis;

(2) A history of close contact within six months of donation with an individual having viral hepatitis;

(3) A history of having received within six months human blood, or any derivative of human blood which the National Institutes of Health has advised the licensed establishment is a possible source of viral hepatitis.

(d) *Therapeutic bleedings.* Blood withdrawn in order to promote the health of a donor otherwise qualified under the provisions of this section, shall not be used as a source of Whole Blood (Human) unless the container label conspicuously indicates the donor's disease that necessitated withdrawal of blood.

(e)¹ *Immunized donors.* Blood withdrawn from donors known to have been immunized to human blood cell antigens shall not be used for Whole Blood (Human) unless the container label conspicuously indicates such information.

§ 73.302¹ Collection of the blood.

(a) *Supervision.* Blood shall be drawn from the donor by a qualified physician or under his supervision by assistants trained in the procedure.

(b)² *The donor clinic.* The pertinent requirements of §§ 73.35 and 73.36 shall apply at both the licensed establishment and at any other place where the bleeding is performed.

¹ Sec. 73.301(e) added and paragraphs (c) and (d) of Sec. 73.302 amended March 9, 1961, 26 FR 2039, to become effective April 8, 1961.

² Sec. 73.302(b) amended June 28, 1961, 26 FR 5755, to become effective July 28, 1961.

(c) *Blood containers.* Blood containers and donor sets shall be pyrogen-free, sterile and identified by lot number. The amount of anticoagulant required for the quantity of blood to be collected shall be in the blood container when it is sterilized. In addition, all container and donor set surfaces that come in contact with blood used in the processing of Heparinized Whole Blood (Human) shall be water repellent.

(d) *The anticoagulant solution.* The anticoagulant solution shall be sterile and pyrogen-free. One of the following formulae shall be used in the indicated volumes:

(1) *Anticoagulant acid citrate dextrose solutions.*

	Solution A	Solution B
Tri-sodium citrate (Na ₃ C ₆ H ₅ O ₇ ·2H ₂ O).....gm..	22.0	13.2
Citric acid (C ₆ H ₈ O ₇ ·H ₂ O).....do.....	8.0	4.8
Dextrose (C ₆ H ₁₂ O ₆ ·H ₂ O).....do.....	24.5	14.7
Water for injection (U.S.P.) to make.....ml.	1,000	1,000
Volume per 100 ml. blood.....do.....	15	25

(2) *Anticoagulant heparin solution.**

Heparin sodium (U.S.P.).....	75,000 units
Sodium chloride injection (U.S.P.) to make..	1,000 ml.
Volume per 100 ml. of blood.....	6 ml.

No other anticoagulant may be used unless prior to use the Surgeon General has found that under such conditions of dating, storage, and use as he may prescribe, the proposed anticoagulant is at least as effective as the above formulae in preventing coagulation and in preserving red blood cells.

(e) *Donor identification.* Each unit of blood shall be so marked or identified by number or other symbol as to relate it to the individual donor.

(f) *Prevention of contamination of the blood.* The skin of the donor at the site of phlebotomy shall be prepared thoroughly and carefully by a method that gives maximum assurance of a sterile container of blood. The blood shall be collected by aseptic methods in a sterile system which may be closed or may be vented if the vent protects the blood against contamination.

(g)¹ *Pilot samples for laboratory tests.* Pilot samples for laboratory tests shall meet the following standards:

(1) One or more pilot samples shall be provided with each unit of blood when issued or reissued

*A buffer suitable to maintain stability to be added, if necessary.

except as provided in § 73.304(e) (2) and all pilot samples shall be from the donor who is the source of the unit of blood.

(2) All samples for laboratory tests performed by the manufacturer and all pilot samples accompanying a unit of blood shall be collected at the time of filling the final container by the person who collects the unit of blood.

(3) All containers for all samples shall bear the donor's identification before collecting the samples.

(4) All containers for pilot samples accompanying a unit of blood shall be attached to the whole blood container before blood collection, in a tamper-proof manner that will conspicuously indicate removal and re-attachment.

(h)¹ *Phlebotomy for Heparinized Whole Blood (Human).* Heparinized Whole Blood (Human) shall be collected with minimal damage to and minimal manipulation of the donor's tissue, and with a single, uninterrupted, free-flowing venipuncture.

(i)¹ *Storage.* Immediately after collection, the blood shall be placed in storage within a 2° range between 1° and 6° C., unless it must be transported from the donor clinic to the processing laboratory. In the latter case the blood shall be placed in temporary storage having sufficient refrigeration capacity to cool the blood continuously toward a 2° range between 1° and 6° C. until it arrives at the processing laboratory where it shall be stored within a 2° range between 1° and 6° C.

§ 73.303² **Testing the blood.**

All laboratory tests shall be made on a pilot sample specimen of blood taken from the donor at the time of collecting the unit of blood, and these tests shall include the following:

(a) *Serological test for syphilis.* Whole Blood (Human) shall be negative to a serological test for syphilis, excepting that blood may be issued in an emergency without performing a serological test for syphilis, provided that the label conspicuously indicates that the test was not done.

(b) *Determination of blood group.* Each container of Whole Blood (Human) shall be classi-

¹ Paragraphs (g), (h), (i), of Sec. 73.302 amended March 9, 1961, 26 FR 2040, to become effective April 8, 1961.

² Sec. 73.303 amended June 28, 1961, 26 FR 5755, to become effective July 28, 1961.

fied as to ABO blood group. At least two blood group tests shall be made and the unit shall not be issued until grouping tests by different methods or with different lots of antisera are in agreement. Only those Anti-A and Anti-B Blood Grouping Serums licensed under, or that otherwise meet the requirements of, the regulations of this part shall be used, and the technique used shall be that for which the serum is specifically designed to be effective.

(c) *Determination of the Rh factors.* Each container of Whole Blood (Human) shall be classified as to Rh type on the basis of tests done on the pilot sample. The label shall indicate the extent of typing and the results of all tests performed. If the test, using Anti-Rh₀ (Anti-D) Typing Serum, is positive, the container may be labeled "Rh Positive". If this test is negative, the results shall be confirmed by further testing which may include tests for the Rh₀ variant (D^u) and for other Rh-Hr factors. Blood may be labeled "Rh Negative" if negative to tests for the Rh₀ (D) and Rh₀ variant (D^u) factors. If the test using Anti-Rh₀ (Anti-D) Typing Serum is negative, but not tested for the Rh₀ variant (D^u), the label must indicate that this test was not done. Only Anti-Rh Typing Serums licensed under, or that otherwise meet the requirements of, the regulations of this part shall be used, and the technique used shall be that for which the serum is specifically designed to be effective.

(d) *Sterility test.*¹ Whole Blood (Human) intended for transfusion shall not be tested for sterility by a method that entails entering the final container before the blood is used for transfusion.

(e) *Inspection.* Whole Blood (Human) shall be inspected visually during storage and immediately prior to issue. If the color or physical appearance is abnormal or there is any indication or suspicion of microbial contamination the unit of Whole Blood (Human) shall not be issued for transfusion.

§ 73.304 General requirements.

(a)² *Manufacturing responsibility.* All manufacturing of Whole Blood (Human), including

donor examination, blood collection, laboratory tests, labeling, storage and issue, shall be done under the supervision and control of the same licensed establishment except that the Surgeon General may approve arrangements, upon joint request of two or more licensed establishments, which he finds are of such a nature as to assure compliance otherwise with the provisions of this part.

(b)³ *Periodic check on sterile technique.* Within the 18th to 24th day after collection, at least one container of blood that upon visual examination appears normal, shall be tested each month as a continuing check on technique of blood collection. The test shall be performed with a total sample of no less than 10 ml. of blood and a total volume of fluid thioglycollate or thioglycollate broth medium 10 times the volume of the sample of blood. The test sample shall be inoculated into one or more test vessels in a ratio of blood to medium of 1 to 10 for each vessel, mixed thoroughly, incubated for seven to nine days at a temperature of 30° to 32° C., and examined for evidence of growth of microorganisms every workday throughout the test period. On the third, fourth, or fifth day at least 1 ml. of material from each test vessel shall be subcultured in additional test vessels containing the same culture medium and in such proportion as will permit significant visual inspection, mixed thoroughly, incubated for seven to nine days at a temperature of 30° to 32° C. and examined for evidence of growth of microorganisms every workday throughout the test period. If growth is observed in any test vessel, the test shall be repeated to rule out faulty test procedure, using another sample of blood from either, (1) the container from which the initial test sample was taken, (2) the residual cells or plasma from that blood, or (3) two different containers of blood, each 18 to 24 days old and each tested separately. The formula for fluid thioglycollate medium shall be as prescribed in § 73.73(e) (1) and the formula for thioglycollate broth medium shall be as prescribed in § 73.73 (f) (5). Media and design of container shall meet the requirements prescribed in § 73.73(e) (2) (i). In lieu of performing one test using an incubation temperature of 30° to 32° C., two tests may be performed, each in all respects as prescribed in

¹ Sec. 73.303(d) amended March 9, 1961, 26 FR 2040, to become effective April 8, 1961.

² Sec. 73.304(a) amended June 28, 1961, 26 FR 5755, to become effective July 28, 1961.

³ Sec. 73.304(b) amended January 11, 1962, 27 FR 307, to become effective February 10, 1962.

this paragraph, one at an incubation temperature of 18° to 22° C. and one at an incubation temperature of 35° to 37° C. A different test may be performed provided that prior to the performance of such a test a manufacturer submits data which the Surgeon General finds adequate to establish that the different test is equal or superior to the test herein prescribed as a check on sterile technique and makes the finding a matter of official record.

(Sec. 215, 58 Stat. 690, as amended; 42 U.S.C. 216. Interpret or apply sec. 351, 58 Stat. 702, as amended; 42 U.S.C. 262)

(c) *Final container.* The original blood container shall be the final container and shall not be entered prior to issue for any purpose except for blood collection. Such container shall be uncolored and transparent to permit visual inspection of the contents and any closure shall be such as will maintain an hermetic seal and prevent contamination of the contents. The container material shall not interact with the contents under the customary conditions of storage and use, in such a manner as to have an adverse effect upon the safety, purity, or potency of the blood.

(d)¹ *Shipment of Whole Blood (Human).* Whole Blood (Human) shall be maintained continuously at 1° to 10° C. during shipment.

(e) *Reissue of blood.* Blood that has been removed from storage controlled by a licensed establishment shall not be reissued by a licensed establishment unless the following conditions are observed:

(1) The container has a tamper-proof seal when originally issued and this seal remains unbroken;

(2) An original pilot sample is properly attached and has not been removed, except that blood lacking a pilot sample may be reissued in an emergency provided it is accompanied by instructions for sampling and for use within six hours after entering the container for sampling;

(3)¹ The blood has been maintained continuously at 1° to 10° C.;

(4) The blood is held for observation until a significant inspection consistent with the requirements of § 73.303(e) can be made.

(f) *Records.* Records shall be maintained of

all aspects of the applicable procedures specified in this part.

§ 73.305 Labeling.

In addition to the items, required in §§ 73.50, 73.51, 73.52, 73.54, and 73.55 or otherwise by law, the following must appear on the label of each container:

(a) *Anticoagulant.* Quantity and kind of anticoagulant used and the volume of blood corresponding with the formula prescribed or approved under § 73.302(d).

(b)¹ *Serological test.* The serological test for syphilis used and the result.

(c) *Blood group and type.* Designation of blood group and Rh factors:

(1) The blood group and Rh factors shall be designated conspicuously.

(2) If a color scheme for differentiating the ABO blood groups is used, the color used to designate each blood group on the container shall be:

Blood Group A : Yellow.

Blood Group B : Pink.

Blood Group O : Blue.

Blood Group AB : White.

(d) *Additional information for labels of Group O Bloods.* Each Group O blood shall be labeled with a statement indicating whether or not isoagglutinin titers or other tests to exclude so-called "dangerous" Group O bloods were performed, and indicating the classification based on such tests.

§ 73.306² Dating period.

The dating period for Citrated Whole Blood (Human) using one of the anticoagulant Acid Citrate Dextrose solutions specified in § 72.302(d) shall be no longer than 21 days after the date of bleeding the donor. The dating period for Heparinized Whole Blood (Human) shall be no longer than 48 hours after the hour of bleeding the donor. The dating period for Whole Blood (Human) using any other anticoagulant found acceptable under such section shall be determined by the Surgeon General on the basis of length of red blood cell survival.

¹ Secs. 73.304(d), 73.304(e)(3) and 73.305(b) amended March 9, 1961, 26 FR 2040, to become effective April 8, 1961.

² Sec. 73.306 amended March 9, 1961, 26 FR 2040, to become effective April 8, 1961.

ADDITIONAL STANDARDS: PACKED RED
BLOOD CELLS (HUMAN)¹

§ 73.320 Proper name and definition.

The proper name of this product shall be Packed Red Blood Cells (Human). The product is defined as red blood cells remaining after separating plasma from sedimented Citrated Whole Blood (Human) to attain an hematocrit reading of from 60 percent to 70 percent.

§ 73.321 Manufacture.

Packed Red Blood Cells (Human) shall be prepared from Citrated Whole Blood (Human) that meets the standards prescribed in §§ 73.301 through 73.304. Packed Red Blood Cells (Human) may be prepared either by centrifugation conducted no later than six days after the date of blood collection or by normal undisturbed sedimentation before the expiration date of the blood. Packed Red Blood Cells (Human) shall be maintained at a temperature between 1° and 10° C. during processing, all of which shall be conducted so as to maintain the sterility of the product. Surfaces of apparatus, including needles and tubing, that come in contact with the product during its processing shall be clean, sterile and pyrogen-free. If the method of processing involves a vented system, the vent shall be one that will exclude microorganisms and maintain a sterile system.

§ 73.322 Pilot samples.

Pilot samples shall meet the following standards:

(a) One or more pilot samples shall be provided with each unit of Packed Red Blood Cells (Human) when issued or reissued except as provided in § 73.304(e) (2).

(b) Pilot samples shall be either the original blood pilot sample or a sample of the Packed Red Blood Cells (Human) being processed.

(c) All containers of pilot samples accompanying a unit of Packed Red Blood Cells (Human) shall be filled at the time the blood is collected or at the time the final product is prepared, by the

person performing the collection or preparation.

(d) All containers for all samples shall bear the donor's identification before they are filled.

(e) All containers for pilot samples accompanying a unit of cells shall be attached securely to the final container of Packed Red Blood Cells (Human) before the final container is filled, in a tamper-proof manner that will conspicuously indicate removal and reattachment.

§ 73.323 Containers.

Final containers used for Packed Red Blood Cells (Human) shall meet the requirements for blood containers prescribed in § 73.304(c) and may be either the original blood containers or different containers.

§ 73.324 Labeling.

Except for the proper name, "Citrated Whole Blood (Human)", labels for Packed Red Blood Cells (Human) shall bear the information required for the Citrated Whole Blood (Human) from which it is processed.

§ 73.325 Expiration date.

The dating period for Packed Red Blood Cells (Human) shall be no longer than 21 days after the date of bleeding the donor for the Citrated Whole Blood (Human) from which it was processed, except that if the hermetic seal is broken during processing the dating period shall be no longer than 24 hours after the seal was broken.

§ 73.326 Storage.

Immediately after processing, the cells shall be placed in storage and maintained within a 2° range between 1° and 6° C.

§ 73.327 General requirements.

Manufacturing responsibilities, periodic check on sterile technique, shipment, reissue and record-keeping shall be carried out in all respects for Packed Red Blood Cells (Human) as prescribed in § 73.304 (a), (b), (d), (e), and (f) respectively for Whole Blood (Human).

¹ Secs. 73.320 through 73.327 added August 13, 1961, 26 FR 7341, to become effective October 11, 1961.

ADDITIONAL STANDARDS: MEASLES IMMUNE
GLOBULIN (HUMAN)¹

§ 73.350 The product.

(a) *Proper name and definition.* The proper name of the product shall be Measles Immune Globulin (Human). It shall consist of a sterile solution of 10 to 18 percent globulin derived from human blood, having a measles antibody level of 0.5 times the level of the NIH measles reference serum. Measles Immune Globulin shall be made from a sterile 16.5 ± 1.5 percent solution of human globulin.

(b) *Source material.* The source of Measles Immune Globulin (Human) shall be blood, plasma or serum from human donors determined at the time of donation to have been free of causative agents of diseases that are not destroyed or removed by the processing method, as determined by the donor's history and from such physical examination and clinical tests as appear necessary for each donor at the time the blood was obtained. The source blood, plasma or serum shall not contain a preservative and shall be stored in a manner that will prevent contamination by microorganisms, pyrogens or other impurities.

(c) *Additives in source material.* Source blood, plasma or serum shall contain no additives other than citrate or acid citrate dextrose anticoagulant solution, unless it is shown that the processing method yields a product free of the additive to such an extent that the safety, purity and potency of the product will not be affected adversely.

§ 73.351 Manufacture of Measles Immune Globulin (Human).

(a) *Processing method.* The globulin shall be prepared by a processing method that (1) has been shown to be capable of concentrating tenfold from source material at least two different antibodies, (2) does not affect the integrity of the globulins and is capable of consistently yielding a product which is safe for subcutaneous and intramuscular injection and (3) will not transmit viral hepatitis.

(b) *Reference materials.* The following reference material shall be obtained from the Division

of Biologics Standards: NIH reference measles serum for correlation of measles antibody titers with globulin products.

(c) *Microbial contamination.* Low temperatures or aseptic techniques shall be used to minimize contamination by microorganisms. Preservatives to inhibit growth of microorganisms shall not be used during processing.

(d) *Bulk storage.* The globulin fraction may be stored in bulk prior to further processing provided it is stored in well-marked hermetically closed containers. Purified globulin as either a liquid concentrate or a solid and containing alcohol or more than 5 percent moisture shall be stored at a temperature not to exceed -10° C. Purified globulin as a solid free from alcohol and containing less than 5 percent moisture, shall be stored at temperatures not to exceed 0° C.

(e) *Determination of the lot.* Each lot of Measles Immune Globulin (Human) shall represent a pooling of material from not less than 1,000 donors.

(f) *Sterilization and dilution.* The product shall be prepared initially as a 16.5 percent solution and this preparation shall be sterilized promptly after solution. After sterilization the product shall not be exposed to temperatures above 45° C. for more than a total of 72 hours. Dilution of this sterile globulin solution shall be made only to adjust the required measles antibody level.

§ 73.352 The final product.

(a) *Final solution.* The final product shall be a 10 to 18 percent solution of globulin containing 0.3 molar glycine and a preservative.

(b) *Protein composition.* No less than 90 percent of the globulin shall have an electrophoretic mobility not faster than -2.8×10^{-5} centimeters per volt per second, when measured at a 1 percent protein concentration in sodium diethylbarbiturate at pH 8.6 and 0.1 ionic strength.

§ 73.353 Potency.

Antibody levels and tests. Each lot of final product shall contain no less than the minimum levels of antibodies for diphtheria and measles as follows:

¹ Secs. 73.350 through 73.354 added 8/1/63, 28 FR 7838.

(a) The product shall contain no less than 2 units of diphtheria antitoxin per ml, adjusted for dilution from the 16.5 percent solution.

(b) Each lot of final product shall contain a measles antibody level of 0.5 times the level of the NIH reference measles serum. The measles antibody potency shall be determined by simultaneous determinations of the neutralizing antibody titers of the globulin on tests and of a reference preparation against 100 TCID₅₀ (50–500 TCID₅₀ when based upon a single test) of measles virus in a tissue culture system. The potency test shall also include a determination of virus titer and controls for globulin toxicity and cell culture viability. Twofold serial dilutions of the globulin under test and of the reference preparation shall be employed in this determination. In applying these requirements a plus or minus variation of one twofold dilution is acceptable.

§ 73.354 General requirements.

(a) *Heat stability test.* Approximately 2 ml of final container material of each lot shall not show any visible sign of gelation after heating in a 12×75 mm. stoppered glass tube at 57° C. for four hours.

(b) *Hydrogen ion concentration.* The pH of final container material shall be 6.8 ± 0.4 when measured in a solution diluted to 1 percent protein with 0.15 molar sodium chloride.

(c) *Turbidity.* The product shall be free of turbidity as determined by visual inspection of final containers.

(d) *Date of manufacture.* The date of manufacture is the date of initiating the last valid measles antibody test as required in § 73.353(b).

(e) *Period of cold storage.* The final product may be held by the manufacturer in cold storage at a temperature not above 5° C. for three years after the date of manufacture without decreasing the length of the dating period. The provisions of § 73.84 shall not apply.

(f) *Dating.* The dating period shall be three years provided the labeling recommends storage from 2–10° C.

(g) *Samples and protocols.* For each lot of globulin, the following materials shall be submitted to the Director, Division of Biologics Stand-

ards, National Institutes of Health, Bethesda, Maryland 20014:

(1) 30 ml of final product.

(2) All protocols relating to the history of the manufacture of each lot and all results of all tests prescribed in these additional standards.

ADDITIONAL STANDARDS: ALLERGENIC PRODUCTS

§ 73.500 The product.

(a) *Definition.* Allergenic Products are products that are administered to man for the diagnosis, prevention or treatment of allergies.

(b) *Criteria for source material.* Only specifically identified allergenic source materials which contain no more than 1 percent of detectable foreign materials, shall be used in the manufacture of an Allergenic Product. Source materials such as feathers, hairs, and danders shall be free from blood and serum.

§ 73.501 Manufacture of Allergenic Products.

(a) *Extraneous allergenic substances.* All manufacturing steps shall be performed so as to insure that the product will contain only the allergenic and other substances intended to be included in the final product.

(b) *Cultures derived from microorganisms.* Culture media into which organisms are inoculated for the manufacture of Allergenic Products shall contain no allergenic substances other than those necessary as a growth requirement. Neither horse protein nor any allergenic derivative of horse protein shall be used in culture media.

(c) *Liquid products for oral administration.* Liquid products intended for oral administration that are filled in multiple dose final containers shall contain a preservative in a concentration adequate to inhibit microbial growth.

(d) *Residual pyridine.* Products for which pyridine is used in manufacturing shall have no more residual pyridine in the final product than 25 micrograms per milliliter.

§ 73.502 Tests.

(a) *Identity.* When a specific identity test meeting the provisions of § 73.75 cannot be performed, the manufacture of each lot shall be

separated from the manufacture of other products in a manner that will preclude adulteration, and records made in the course of manufacture shall be in sufficient detail to verify the identity of the product.

(b) *Safety.* A safety test shall be performed on the contents of a final container of each lot of each product as prescribed in § 73.72 except for the following:

(1) For lots consisting of no more than 20 final containers or 20 sets of individual dilutions, or where the final container contains no more than one intended human dose, the safety test need not be performed on the contents of a final container provided the safety test is performed on each lot of stock concentrate and on each lot of diluent contained in the final product. Only stock concentrates and diluents which have passed the general safety test shall be kept in the work areas used for the manufacture of Allergenic Products. A stock concentrate is an extract derived from a single allergenic source and used in the manufacture of more than one lot of product, and from which final dilutions or mixtures are prepared directly.

(2) For powders for scratch tests, a sample shall be suspended in a suitable diluent and injected into each animal, and the sample size shall be the single human dose recommended.

(c) *Sterility.* A sterility test shall be performed on each lot of each Allergenic Product as prescribed in § 73.73, with the following exceptions:

(1) When bulk material is not prepared, the sterility test prescribed for bulk material shall be performed on each container of each stock concentrate at the time a stock concentrate is prepared, and the test sample shall be no less than 1 ml. from each stock concentrate container.

(2) For lots consisting of no more than 5 final containers, the final container test shall be performed in accordance with § 73.73(f) (7) using the sample therein prescribed or using a sample of no less than 0.25 ml. of product from each final container, divided in approximately equal proportions for testing in Fluid Thioglycollate and Fluid

Sabouraud's media. The test sample in the latter alternative method may be an overfill in the final container.

(3) For products prepared in sets of individual dilution series, a test sample of 0.25 ml. shall be taken from a final container of each dilution, which samples may be pooled and one half of the pooled material used for the test with fluid Thioglycollate medium and one-half used for the test with fluid Sabouraud's medium.

(4) Tablets and capsules need not be tested for sterility provided aseptic techniques are employed in their manufacture.

§ 73.503 Dating.

(a) *Periods of cold storage.* Allergenic Products may be held by the manufacturer in cold storage after the date of manufacture without decreasing the dating periods prescribed in paragraph (b) of this section, as follows:

(1) Products with 50 percent or more glycerin and freeze-dried products, at not above 5 degrees C. for three years.

(2) Products with less than 50 percent glycerin, at not above 5 degrees C. for eighteen months.

(b) *Dating periods.* The dating periods for Allergenic Products shall not exceed the following:

(1) Products with 50 percent or more of glycerin, three years, provided labeling recommends storage from 2 degrees C. to 8 degrees C.

(2) Products with less than 50 percent glycerin, eighteen months, provided labeling recommends storage from 2 degrees C. to 8 degrees C.

(3) Liquid products for which refrigerator temperatures are inappropriate, eighteen months, provided labeling recommends storage at not above 30 degrees C. § 73.84 and paragraph (a) of this section do not apply.

(4) Powders and tablets: Five years, provided labeling recommends storage at not above 30 degrees C. § 73.84 and paragraph (a) of this section do not apply.

(5) Freeze-dried products: Five years provided labeling recommends storage from 2 degrees C. to 8 degrees C.



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